Immunoassay of Plasma Insulin in Man

Rosalyn S. Yalow, Ph.D., and Solomon A. Berson, M.D., New York*

In recent years, considerable effort has been directed toward the establishment of a sensitive and reliable assay for insulin in body fluids.[1] A detailed review of work in this field is clearly outside the confines of this brief presentation. For present purposes, it is sufficient to note that many of the methods which depend on a biologic response in vitro or in vivo suffer from a lack of sensitivity or specificity and a susceptibility to interfering substances when applied to assay of the very minute concentrations of endogenous insulin in blood.

The immunoassay to be described appears to be free of interference from noninsulin substances in blood yet is sufficiently sensitive to require only 0.01 ml. plasma, or less, in routine applications. The principle of the immunoassay is based on earlier observations that binding of I^{131}-labeled insulin to insulin antibodies is competitively inhibited in the presence of unlabeled insulin.[2] Thus, a quantitative relationship could be established between the ratio (B/F) of antibody-bound insulin-I^{131} (B) to unbound, "free," insulin (F) and the concentration of unlabeled insulin in solutions prepared from known standards, thereby permitting determination of insulin in unknown samples.[3-8] Although antibody-bound and free insulin may be separated by a number of technics,[2,9-12] we have continued to employ the method of hydrodynamic flow paper strip chromatography,[2] preferred for its precision and economy of effort in the handling of multiple samples and extraordinary sensitivity. Separation of bound and free insulin by this technic depends on the firm adsorption of free insulin to the paper at the site of application ("origin")[2,13] and the migration of antibody-bound insulin away from the origin with the other serum proteins.[2] In the presence of insulin-I^{131}, two radioactive peaks are produced, one at the origin representing free insulin, the other with the serum proteins, representing antibody-bound insulin.[2]†

Figure 1 shows a set of chromatograms, a plot of the B/F ratios for pork insulin-I^{131} as a function of human insulin concentration in known standards and a sample calculation for the insulin concentration in an unknown sample. In previous experiments less than one microunit (37 $\mu\mu$g) insulin was readily measurable.[8] In more recent studies, this sensitivity has been increased significantly permitting detection of less than 0.1 μU insulin (figure 1). As many as 300 or more unknown samples have been processed simultaneously with these technics.

It has been shown that human insulin added to plasma is recovered quantitatively and that on dilution of plasma, the measured endogenous insulin concentration decreases proportionately, indicating the absence of plasma inhibitors of the reaction.[6] Furthermore, cysteine, which inactivates insulin-I^{131} in plasma, and cellulose, which removes insulin-I^{131} from plasma by adsorption, have similar effects on endogenous insulin measured by immunoassay.[6]

The species-specificity of the insulin-antibody reaction[15] requires that standard solutions be prepared from insulin of the same species as is present in unknown samples although the I^{131}-labeled insulin may be derived from any species. Thus, human insulin must be employed as a standard for the assay of endogenous insulin in man. For the most part, guinea pig antiserums will discriminate among different species of insulin and, in particular, human insulin generally reacts less strongly than beef insulin as a competitive inhibitor against the binding of beef insulin-I^{131} by guinea pig anti-beef insulin serums.[6] However, we have recently observed that serums from guinea pigs immunized with pork insulin usually show quite similar reactions with pork and human insulin and we have obtained two antiserums (one of which is shown in figure 2) which react identically with these insulins[16] so that future studies will not be dependent on availability of the relatively scarce supplies of human insulin.

Inasmuch as insulin-binding antibodies uniformly present in serums of insulin-treated subjects[2] may interfere with the immunoassay, our efforts, heretofore, had been restricted to assay of plasma insulin in subjects never previously treated with insulin.

Presented at the Twenty-first Annual Meeting of the American Diabetes Association in New York City on June 24, 1961.

From the Radioisotope Service, Veterans Administration Hospital, Bronx 68, New York.

*Winner of the Lilly Award of the American Diabetes Association for 1961.

†On electrophoresis, antibody-bound insulin migrates between γ and β globulins.[2,14]

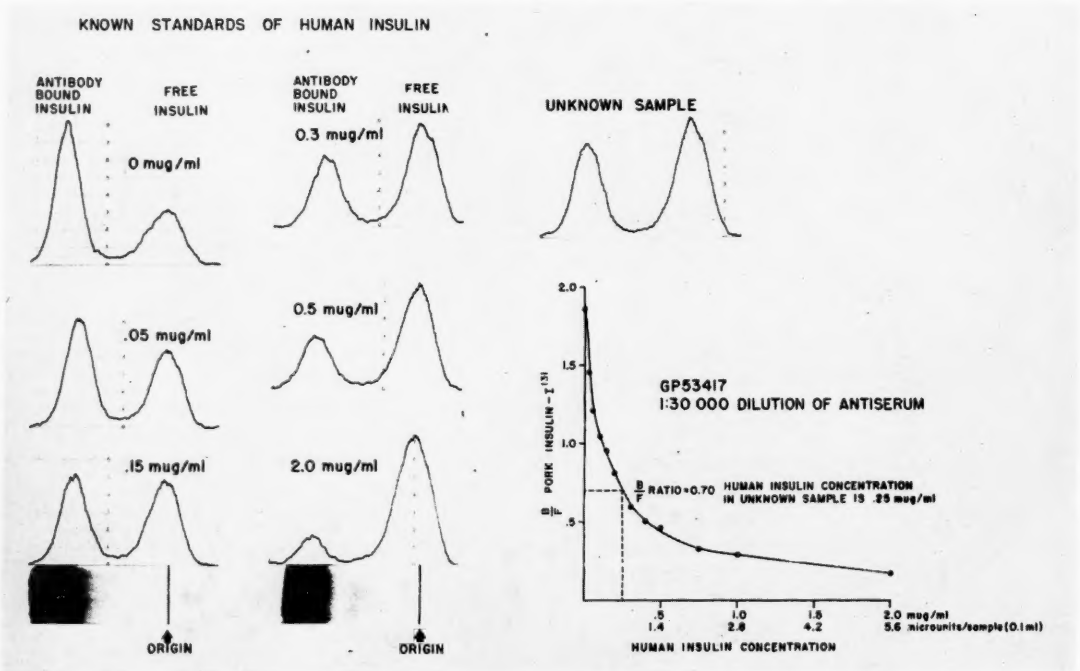


FIG. 1. Radio-chromato-electrophoretograms (left) of insulin-antiserum mixtures prepared for standard curve (lower right). All standard solutions contained the same concentration of pork I^{131}-insulin and of guinea pig antiserum (final dilution—1:30,000). The concentration of unlabeled human insulin in mixtures varied as indicated. The standard curve was obtained from planimetric measures of areas under each of the two peaks in the complete series of chromatograms, six of which are shown. Also shown is the chromatogram of an unknown serum sample (at a final dilution of 1:10) in a mixture containing the same concentrations of pork I^{131}-insulin and antiserum as in standard solutions. From the measured B/F ratio of 0.70 in this chromatogram, the insulin concentration is calculated as shown (lower right).

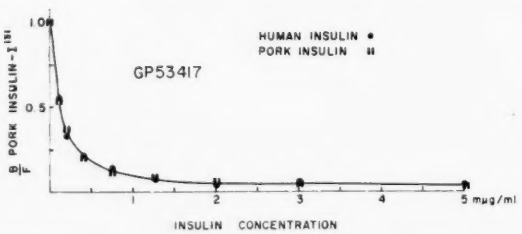


FIG. 2. B/F (Pork insulin-I^{131}) as a function of concentration of pork insulin or human insulin. Pork and human insulin react identically competing against pork insulin-I^{131} in this guinea pig antipork insulin serum.

Plasma insulin concentrations in patients with early maturity-onset diabetes, previously untreated or treated only with sulfonylureas, are compared with those of nondiabetic subjects in the fasting state and during the course of a standard 100 gm. oral glucose tolerance test (figure 3). Although the nondiabetic subjects showed, on the average, a more brisk early insulin-secretory response, most of the diabetic patients revealed the capacity to secrete even greater amounts of insulin than were sufficient to restore the *normal* subject rapidly to a euglycemic state. Thus, the diabetic exhibits much higher than normal amounts of insulin at one and two hours which, for reasons as yet unknown, fail to induce a normal reduction in blood sugar. That the response of the early diabetic in these instances does not represent an extreme, agonizing effort of the islet tissue is indicated by the still very much higher plasma insulin levels that can be achieved in these patients under the stimulus of heavier glucose loading[6] (figure 3). We have concluded from these observations that the early diabetic does not respond to his insulin as well as a normal subject does to his insulin.[6,8]

Tolbutamide administration is almost invariably followed by an increase in peripheral blood insulin in both normal and early diabetic states, but the response is generally very much less impressive than that which follows glucose by the same route of administration.[17]

Plasma insulin concentrations in patients with functioning islet-cell adenoma may be quite variable. In

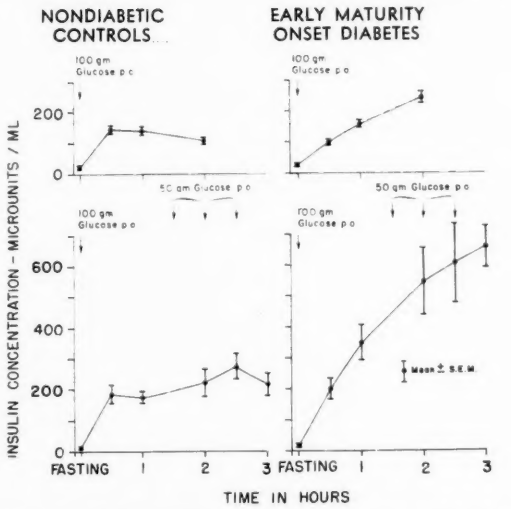


FIG. 3. (Top) Mean plasma insulin concentration before and during a standard 100 gm. oral glucose tolerance test in subjects never treated with insulin (thirty nondiabetic subjects and thirty-eight diabetic subjects). (Bottom) Same as above except that additional loads of 50 gm. glucose were administered during the test at 1½, 2 and 2½ hours. (Four diabetic subjects and five nondiabetic subjects.)

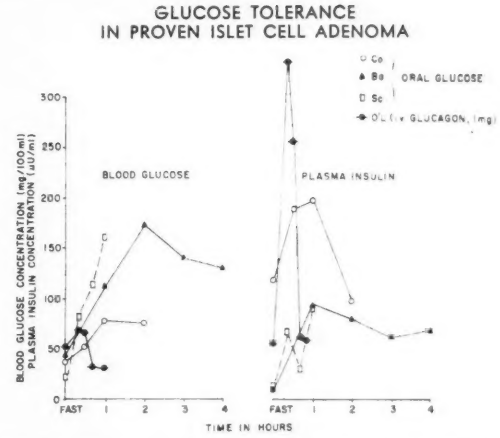


FIG. 4. Blood glucose and plasma insulin concentrations after glucose (100 gm. p.o.) or glucagon (1 mg. i.v.) administration in patients with proven islet-cell adenoma.

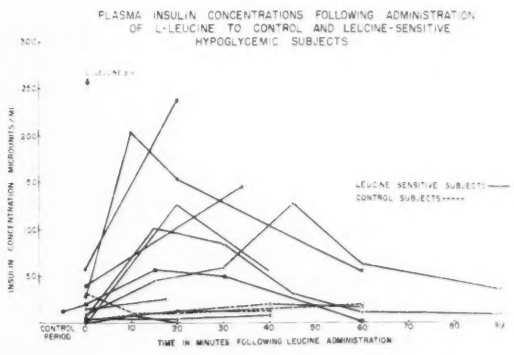

PLASMA INSULIN CONCENTRATIONS FOLLOWING ADMINISTRATION OF L-LEUCINE TO CONTROL AND LEUCINE-SENSITIVE HYPOGLYCEMIC SUBJECTS
LEUCINE SENSITIVE SUBJECTS
CONTROL SUBJECTS
CONTROL PERIOD
TIME IN MINUTES FOLLOWING LEUCINE ADMINISTRATION

FIG. 5. 75-150 mg./kg. L-leucine administered.

about half of the cases the fasting insulin concentration, at the time of hypoglycemia, is significantly higher than normal.[6] On occasion, a significant elevation of plasma insulin has not been detected over a period of several hours of prolonged hypoglycemia. It may be that even moderate insulin concentrations in the presence of persistent hypoglycemia should be regarded as evidence of improperly controlled insulin secretion but it is difficult to interpret the failure of counter-regulatory restoration of blood sugar in the absence of *significant* hyperinsulinism during these periods. From the relatively poor glucose tolerance associated with a comparatively meager output of insulin in some of the patients with islet-cell adenoma (figure 4), it may be deduced that the tumor itself is not particularly sensitive to stimulation by glucose itself and, further, that the normal islet tissue may be suppressed by insulin from the tumor.

We have also examined plasma insulin in patients with idiopathic hypoglycemia who develop a striking fall in blood sugar acutely following ingestion of L-leucine, a syndrome first recognized by Cochrane et al.[18] The typical response is an acute elevation in plasma insulin concentration (figure 5) concomitant with the fall in blood sugar, but an inexplicable feature of this disorder is the frequent presence of fasting hypoglycemia not associated with significant hyperinsulinism.[6] Since *toxic* doses of L-leucine can induce profound hypoglycemia even in normal rats,[19] it is hypothetically conceivable that the islet-cell of the leucine-sensitive hypoglycemic patient has a lower than normal threshold for response to both leucine and glucose. A somewhat similar abnormality may underlie the occasional exaggerated response of islet-cell adenomata to leucine[20-22] or tolbutamide[23] since moderate or marked increases in plasma insulin have been observed in association with the hypoglycemia following leucine[22,21] and tolbutamide (figure 6). Although glucose tolerance may be poor in some patients with islet-cell tumors, the insulin-secretory response to tolbutamide may still be greater than is usually observed in normal or diabetic subjects.[17] However, the prolonged hypoglycemia following tolbutamide first observed by Fajans et al.[23] and also evident here in Case Be is not readily explained. Here, too, as in idiopathic hypoglycemia, there appears to be a defect in

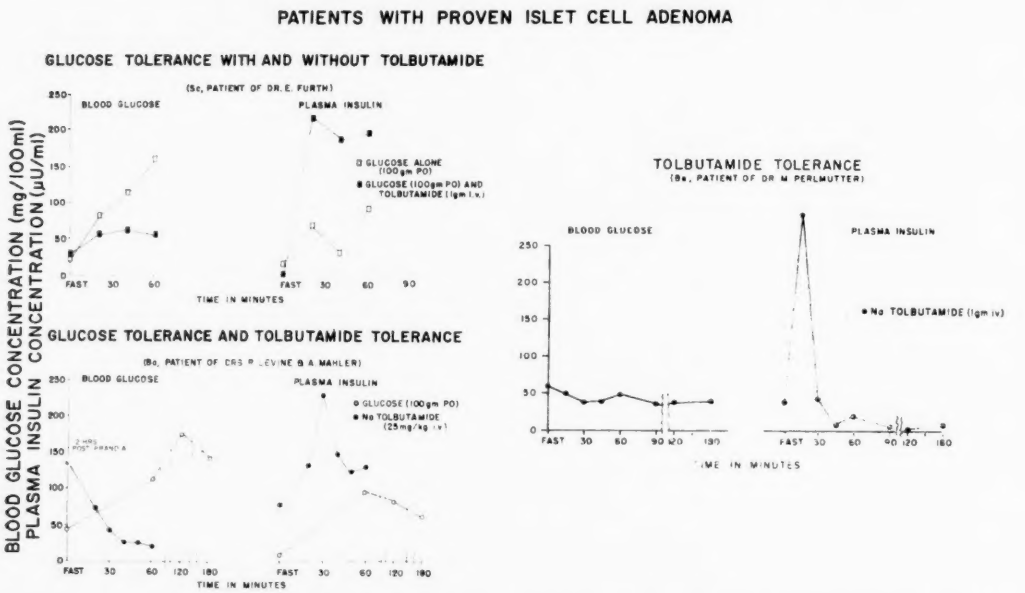

PATIENTS WITH PROVEN ISLET CELL ADENOMA
GLUCOSE TOLERANCE WITH AND WITHOUT TOLBUTAMIDE
(Sc, PATIENT OF DR. E. FURTH)
BLOOD GLUCOSE
PLASMA INSULIN
GLUCOSE ALONE (100 gm PO)
GLUCOSE (100gm PO) AND TOLBUTAMIDE (1gm I.v.)
BLOOD GLUCOSE CONCENTRATION (mg/100ml)
PLASMA INSULIN CONCENTRATION (μU/ml)
FAST 30 60 FAST 30 60 90
TIME IN MINUTES
GLUCOSE TOLERANCE AND TOLBUTAMIDE TOLERANCE
(Bo, PATIENT OF DRS. P. LEVINE & A. MAHLER)
BLOOD GLUCOSE
PLASMA INSULIN
GLUCOSE (100gm PO)
No TOLBUTAMIDE (25mg/kg i.v.)
FAST 30 60 120 180 FAST 30 60 120 180
TIME IN MINUTES
TOLBUTAMIDE TOLERANCE
(Be, PATIENT OF DR. M. PERLMUTTER)
BLOOD GLUCOSE
PLASMA INSULIN
No TOLBUTAMIDE (1gm iv)
FAST 30 60 90 120 180 FAST 30 60 90 120 180
TIME IN MINUTES

FIG. 6. Blood glucose and plasma insulin concentrations following administration of glucose, tolbutamide or glucose plus tolbutamide, as indicated, to patients with proven islet-cell adenoma.

counter-regulatory restoration of blood sugar not attributable to persistent hyperinsulinism.

Finally, a brief report on our recent determination of plasma insulin in insulin-treated patients is presented. Several difficulties beset the determination of plasma insulin in such patients. The first problem is unique to the immunoassay method and results from some binding by endogenous insulin antibody of the tracer insulin-I^{131} meant for the guinea pig antiserum thereby confusing the assay. Two other complications are common to all methods of insulin assay although consideration of them has generally been neglected. Since a variable fraction of administered insulin binds to endogenous antibody to a degree determined by the concentration of antibody as well as by a number of other factors, and since antibody-bound insulin probably has a half life not much shorter than a day or so, it is evident that the amount of exogenously administered insulin remaining in the blood stream, even after three to five days, may exceed the amount of endogenous insulin present. Thus, if a patient cannot tolerate insulin withdrawal for a sufficient period of time, in many cases a reliable determination of his own plasma insulin is prohibited with any method of assay.

Further, even when prolonged withdrawal of insulin can safely be effected, some fraction of endogenous insulin will generally be bound to endogenous antibody, and that which does not dissociate in vitro will not be free to act on tissues in bioassay systems or to compete with labeled insulin in the immunoassay system.

However, these difficulties have recently been overcome by exploiting the marked difference in size between insulin and antibody and their separation on ultracentrifugation of plasma. The relatively low sedimentation velocity of insulin permits it to remain in the supernatant above the serum albumin boundary, whereas the antibody sediments more rapidly than the albumin. The loss of free insulin from the supernatant as a result of sedimentation is determined from analysis of insulin-I^{131} concentration in the supernatant of control (nonimmune) plasma, centrifuged simultaneously. Immunoassay of endogenous insulin concentration in the supernatant of unknown plasma then yields a value for total free endogenous plasma insulin under the conditions of dilution and temperature used for sedimentation. From simultaneous chromato-electrophoretic determination of the ratio between bound and free human insulin-I^{131} in the same serum under the same conditions, the plasma concentration of bound and total endogenous insulin is obtained.

Alternatively, because of the great sensitivity of the immunoassay procedure, the insulin concentration may be assayed in plasma diluted as much as 1:50. By taking

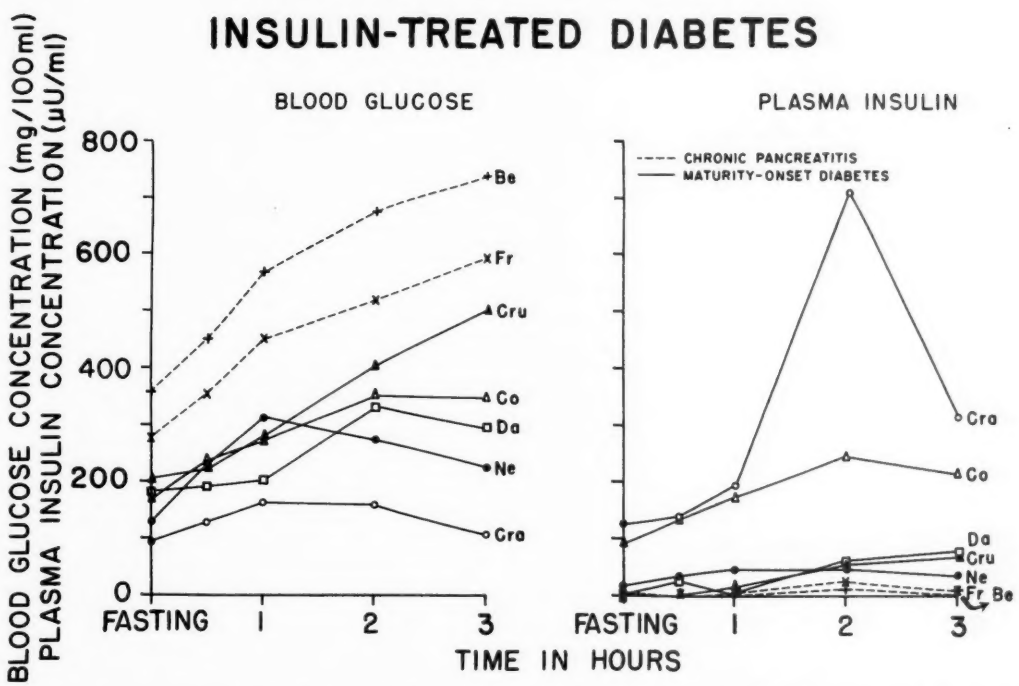


FIG. 7. Blood glucose and plasma insulin concentrations in diabetic subjects previously treated with insulin. Co was receiving tolbutamide therapy at time of study. The total plasma insulin concentrations were determined from immunoassay of the supernatant fraction following ultracentrifugation. Correction for sedimentation of free insulin and for binding of endogenous human insulin by endogenous antibody as described in text. In patient Co plasma insulin concentrations determined from a 1:50 dilution of uncentrifuged plasma using human insulin-I^{131} (Fasting, 126μU/ml.; ½ hr., 154μU/ml.; 1 hr., 189μU/ml.; 2 hr., 210μU/ml.; 3 hr., 217μU/ml.) were in fair agreement with those shown in this figure.

advantage of the weaker binding of human than of animal insulin to human antibodies, it is occasionally possible, even in the presence of endogenous antibody, to perform the immunoassay with human insulin-I^{131} using sufficiently diluted plasma. Since these methods are more onerous than those available for nontreated subjects, available data are rather meager. Some results are shown in figure 7. The virtual absence of insulin in the severe diabetics is evident. The relatively high total plasma insulin concentrations in the mild diabetic (Cra) may be interpreted in terms of the partial sequestration by antibody, in consequence of which, available free insulin is reduced.

Finally, it must be emphasized that the total insulin concentration in subjects with antibody may not have the same biologic significance as in untreated subjects. The availability of free endogenous insulin for tissue utilization under in vivo conditions will depend upon the concentration of antibody and the rates of association and dissociation of endogenous insulin and antibody compared to the rate of egress of free insulin from the blood stream as well as on other factors.

SUMMARY AND CONCLUSIONS

Recent work on the immunoassay of plasma insulin in man has been reviewed. The sensitivity of the method has been increased to 0.1 μU insulin and new antiserums from pork insulin-immunized guinea pigs have been obtained which react identically with pork and human insulins, thereby obviating the requirement for human insulin standards. Methods applicable to the determination of plasma insulin in insulin-treated subjects are also presented and data on plasma insulin levels in various hypoglycemic and hyperglycemic states are summarized.

SUMMARIO IN INTERLINGUA

Immuno-Essayage del Insulina Plasmatic in le Homine

Es revistate le recente labores in re le immuno-essayage del insulina plasmatic in le homine. Le sensibilitate del

methodo ha essite augmentate usque a 0,1 μU de insulina, e nove antiseros ab porcos de India immunisate con insulina porcin e human, de maniera que le requirimento de standards de insulina human es obviate. Es etiam presentate methodos applicabile al determination de insulina plasmatic in subjectos tractate con insulina, e datos relative al nivellos plasmatic de insulina in varie statos hypo- e hyperglycemic es summarisate.

REFERENCES

[1] Yalow, R. S., and Berson, S. A.: Editorial: Plasma insulin in man. Am. J. Med. *29*:1, 1960.

[2] Berson, S. A., Yalow, R. S., Bauman, A., Rothschild, M. A., and Newerly, K.: Insulin-I^{131} metabolism in human subjects. Demonstration of insulin binding globulin in the circulation of insulin-treated subjects. J. Clin. Investigation *35*:170, 1956.

[3] Berson, S. A., and Yalow, R. S.: Isotopic tracers in the study of diabetes. New York, Academic Press, Inc. Adv. in Biol. and Med. Phys. *6*:349, 1958.

[4] Berson, S. A., and Yalow, R. S.: Recent studies on insulin-binding antibodies. Bull. New York Acad. Sc. *82*:338, 1959.

[5] Yalow, R. S., and Berson, S. A.: Assay of plasma insulin in human subjects by immunological methods. Nature *184*: 1648, 1959.

[6] Yalow, R. S., and Berson, S. A.: Immunoassay of endogenous plasma insulin in man. J. Clin. Investigation *39*:1157, 1960.

[7] Berson, S. A., and Yalow, R. S.: Immunoassay of insulin. Boston, Little, Brown and Co. Hormones in Plasma. Chap. III, *86*:1960.

[8] Yalow, R. S., and Berson, S. A.: Plasma insulin concentrations in nondiabetic and early diabetic subjects. Diabetes *9*:254, 1960.

[9] Skom, J. H., and Talmage, D. W.: The role of non-precipitating insulin antibodies in diabetes. J. Clin. Investigation *37*:787, 1958.

[10] Gordis, E.: Detection of insulin-binding antibodies and separation of free and antibody-bound insulin by rapid chemical procedure. Proc. Soc. Exper. Biol. & Med. *103*:542, 1960.

[11] Moloney, P. J., and Aprile, M. A.: Neutralization of cod insulin with antiserum: Precipitation of insulin-anti-insulin complex with ethanol-water. Canad. J. Biochem. & Physiol. *38*:1216, 1960.

[12] Grodsky, G. M., and Forsham, P. H.: An immunochemical assay of total extractable insulin in man. J. Clin. Investigation *39*:1070, 1960.

[13] Kallee, E.: Über^{131} J-signiertes insulin. I. Mitteilung (Nachweis) Ztschr. Naturforsch *7b*:661, 1952.

[14] Berson, S. A., and Yalow, R. S.: Ethanol fractionation of plasma and electrophoretic identification of insulin-binding antibody. J. Clin. Investigation *36*:642, 1957.

[15] Berson, S. A., and Yalow, R. S.: Species-specificity of human anti-beef, pork insulin serum. J. Clin. Investigation *38*:2017, 1959.

[16] Yalow, R. S., and Berson, S. A.: Immunologic specificity of human insulin: Application to immunoassay of insulin. J. Clin. Investigation. In press.

[17] Yalow, R. S., Black, H., Villazon, M., and Berson, S. A.: Comparison of plasma insulin levels following administration of tolbutamide and glucose. Diabetes *9*:356, 1960.

[18] Cochrane, W. A., Payne, W. W., Simpkiss, M. J., and Woolf, L. I.: Familial hypoglycemia precipitated by amino acids. J. Clin. Investigation *35*:411, 1956.

[19] Gullino, P., Winitz, M., Birnbaum, S. M., Cornfield, J., Oley, M. C., and Greenstein, J. P.: The toxicity of individual essential amino acids and their diastereomers in rats and the effect on blood sugar levels. Arch. Biochem. & Biophys. *58*: 253, 1955.

[20] Schwartz, T. B., Flanagan, G. C., Stuppy, G. W., and Tarum, D. W.: The hypoglycemic effect of L-leucine in organic hyperinsulinism. J. Lab. & Clin. Med. *54*:944, 1959 (abst.).

[21] Flanagan, G. C., Schwartz, T. B., and Ryan, W. G.: Studies on patients with islet-cell tumor, including the phenomenon of leucine-induced accentuation of hypoglycemia. J. Clin. Endocrinol. & Metab. *21*:401, 1961.

[22] Marks, J. F., and Klein, R.: The effect of leucine on plasma insulin concentration in a patient with a pancreatic adenoma. Submitted for publication.

[23] Fajans, S. S., Schneider, J. M., Schteingart, D. E., and Conn, J. W.: The diagnostic value of sodium tolbutamide in hypoglycemic states. J. Clin. Endocrinol. & Metab. *4*:371, 1961.

[24] Berson, S. A., and Yalow, R. S.: Immunoassay of insulin. Ciba Colloquium on "Detection and assay of hormones by immunochemical means." In press 1961.

Influence of Obesity on Morbidity and Mortality

Middle-aged overweight diabetics have shown a return to normal glucose tolerance with reduction in weight. Newburgh demonstrated that where the weight was brought down to the normal level, normal glucose tolerance was restored to nearly 75 per cent of the cases, and improved tolerance was observed in half the remaining cases, as well as in some patients with lesser degrees of weight reduction. Osserman and Dolger obtained roughly comparable results with obese diabetics whose weight was reduced.

A recent study by Goodman of 135 elderly obese patients in a Cleveland Nursing Home in whom diabetes, gangrene, congestive heart failure and cerebral vascular accidents were in excess of the expected number showed that beneficial results were always seen with weight reduction. In 75 per cent of these patients the blood pressure was above 150/90, and the blood pressure levels almost invariably fell with weight reduction.

By Herbert H. Marks in *Bull. New York Acad. Med.*, Second Series, Vol. 36, No. 5, p. 310, May 1960.

Lipoprotein Patterns in Diabetes and the Changes Occurring during Pregnancy

A. Vernet, M.D., and Elspeth B. Smith, Ph.D., London, England

During the last few years there have been numerous studies on plasma lipids and lipoproteins in relation to coronary artery disease. Oliver and Boyd[1] and other authors have shown the presence of hypercholesterolemia, an elevated cholesterol/phospholipid ratio and increased cholesterol in the β-lipoprotein fraction. In patients with myocardial infarction Lindgren and Gofman[2] showed by ultracentrifugation an increase in the low density lipoproteins, particularly the S_f12-400 groups, and Dangerfield and Smith[3] using paper electrophoresis drew attention to a "pre-β-lipid" (p-β-l) band. Besterman[4] found a high p-β-l level in 198 of 200 patients with active coronary artery disease (C.A.D.) and concluded that it was a more specific indicator of atheroma than other lipid measurements. Further studies by Smith[5] showed that it corresponded to the ultracentrifugal S_f20-100 lipoproteins, and that it increased from a low level immediately following coronary occlusion to a maximum three to six weeks after the attack; Dodds and Mills,[6] using both paper electrophoresis and ultracentrifugation, confirmed this finding.

These observations seem compatible with the findings of the Cooperative Study of Lipoproteins and Atherosclerosis[7] in which it was not possible to demonstrate a significant correlation between elevated S_f12-20 and 20-100 lipoproteins and development of new coronary artery disease (C.A.D.) during the next two years. Lawry et al.[8] found marked elevation of these groups in 198 men examined after myocardial infarction but little change in eight cases examined before a new attack. Page[9] found an increase in -S70-400 and 40-70 lipoproteins (equivalent to S_f20-100 and 12-20) in some but not all cases examined during the first few weeks after infarction; in a group of eighteen men examined yearly who developed coronary occlusion both S_f12-20 and 20-100 lipoproteins showed considerable fluctuations, but did not seem to lie outside the range for the base population in the Cooperative Study.

From the Institute of Clinical Research, The Middlesex Hospital, and the Courtauld Institute of Biochemistry, The Middlesex Hospital Medical School, London, W.1, England.

There is thus considerable evidence that a high p-β-l (or S_f12-100) level may be secondary to ischemic lesions of the heart rather than a factor in the pathogenesis of atherosclerosis, and it seemed that some further information might be obtained by examining a group without clinical heart disease, but with a high incidence of coronary atherosclerosis.

Both clinical and postmortem studies have shown an increased incidence of ischemic heart disease and atherosclerosis in diabetics. Angina or infarction has been reported in 17 per cent of women and 27 per cent of men (Lewis and Symons[10]) and in 15 per cent of women and 24 per cent of men (Liebow et al.[11]) in two series of diabetics covering a wide age range. The latter authors used the same criteria for diagnosis as in the Framingham study[12] which examined the incidence of coronary disease in a normal population; in the age group thirty to forty-four years the rate for men was 0.3 per cent, and for women 0.4 per cent; these rates rise to 3.5 per cent and 1.2 per cent in the forty-five- to sixty-two-year age group. The differences observed in postmortem studies are less remarkable; in the age group forty to sixty years Lisa et al.[13] found severe coronary arteriosclerosis in 37 per cent of diabetics and 22 per cent of controls; in older cases the difference became smaller in coronary arteries and aorta but not in the cerebral arteries. Stearns et al.[14] examined the coronary tree and myocardium in subjects over forty-two years of age; the incidence of occlusive coronary disease in diabetic men and women was 68 per cent and 61 per cent and that in the control series 31 per cent and 8 per cent. Bell[15] reports smaller differences in deaths from coronary disease in older people (sexes not differentiated) but in the twenty- to forty-year age group the rate for diabetics was six times that for nondiabetics. Most authors agree that the amount of atherosclerosis is more closely associated with the age of the patient and duration of the diabetes than with its severity.

From these findings it seemed reasonable to assume that diabetics in whom the duration of the disease was more than five years would have more atherosclerosis

than nondiabetics, and that they would provide a suitable group for this study. At the same time we examined a few sera from pregnant diabetic women and were surprised to find some very large p-β-1 bands. This led to investigation of a larger group of pregnant women in order to compare normals and diabetics, and pregnancies following different clinical courses.

MATERIALS AND METHODS

Group A: Diabetic men and women. Forty-eight men and twenty-eight women were examined. More men were selected than women because they are more prone to ischemic heart disease. Nine men and four women had a history of C.A.D. (angina pectoris or myocardial infarction) but at the time of the investigation it was active only in three men.

Group B: Pregnant women. Thirty-four pregnant diabetic women were examined; only two had signs of retinopathy, and in both the pregnancy was uneventful. The diabetic women included: (a) twenty-six successful uncomplicated pregnancies with no signs of toxemia, and normal deliveries. Average age thirty years; average duration of diabetes, eight and one-half years.

(b) Eight unsuccessful or complicated pregnancies. Average age twenty-six years; average duration of diabetes ten years. These consisted of: (1) one patient with severe toxemia at the end of pregnancy in which the baby finally survived; (2) five stillbirths at the 29th, 30th, 34th, 35th and 37th weeks; three of these were preceded by threatened abortion about the twentieth week; (3) two pregnancies terminating with premature babies (weight about five pounds) which died after a day from pulmonary hyaline membrane disease.

The control subjects were fifty-two normal pregnant women, average age 26.5 years. None showed signs of pretoxemia or glycosuria, and all gave birth to normal babies. It was possible to take only one blood sample from each woman, but they were spread over different stages of pregnancy and the puerperium.

Paper electrophoresis was done by the method of Dangerfield and Smith.[3] The p-β-1 was scored by the method of Smith[5] taking into account the extent of the band (scored from 0 to 3) and the intensity of staining, subjectively scored from 0 to 4; this gives a total score from 0 to 7. This score has been used partly because the Sudan black stain is not quantitative, and mainly because the p-β-1 band is overlapped by the front of the β-lipoprotein which makes allocation of the relevant area under scanning curves, or cutting out of fractions for elution, subject to serious error. The score system seems to give a reasonable assessment of the p-β-1 level without implying a greater accuracy than is justified. The score is not generally considered suitable for statistical analysis, but in a group of sixty-three subjects it gave a high degree of correlation ($r = 0.875$) with the S_f20-100 lipoprotein measured in the analytical ultracentrifuge; from the regression coefficient one p-β-1 unit was equivalent to 25 mg./100 ml., but the p-β-1 was not detected below about 40 mg./100 ml.

Serum cholesterol was determined by a modification of the method of Bloor.[16]

An attempt was made to measure the total β-lipoprotein by a modification of the turbidimetric method of Bernfeld et al.[17] using precipitation by dextran sulphate (Oncley et al.[18]).

RESULTS

Group A. The results obtained are set out in table 1 which includes for comparison the p-β-1 and cholesterol levels in comparable groups of nondiabetic subjects.

The dextran sulphate turbidity estimations have not been of value; on a group basis they correlate slightly better with p-β-1 than with total cholesterol, but in individual sera there is a very large scatter.

The serum cholesterol in diabetics without clinical signs of atherosclerosis is rather higher than the normal level, but in groups with clinical atherosclerosis there is little difference between diabetics and nondiabetic controls.

The p-β-1 level in patients with uncomplicated diabetes differs little from that found in normal subjects of comparable ages. Men with coronary artery disease had a slightly increased mean p-β-1 score; in two patients with active angina and one with recent myocardial infarction the mean score was 3.0, whereas in six patients in whom the disease was inactive the mean score was 1.9. The nondiabetic control groups given in table 1 for both men and women with C.A.D. are patients with active angina; in eight men with old infarcts and no angina the mean score was 2.1.

In some patients with diabetic retinitis the p-β-1 is increased; this is particularly marked in the four younger men of whom two had large and two moderate bands. In the eight older men, only one had a large p-β-1.

The number of diabetic men with minimal, moderate or large p-β-1 bands is shown in figure 1. The distribution is very similar to that in comparable nondiabetic subjects (Smith[5]).

Group B. The average cholesterol and p-β-1 values in

TABLE 1

Serum cholesterol, dextran sulphate turbidity and pre-β-lipid in diabetic patients and controls

	Number of cases	Average age	Average duration of diabetes (years)	Serum cholesterol (mg./100 ml.)	Dextran sulphate turbidity (extinction units) (Controls 5-10 u.)	P-beta-1 score	Comparable nondiabetics: Number of subjects	Comparable nondiabetics: Average age	Comparable nondiabetics: Serum cholesterol (mg./100 ml.)	Comparable nondiabetics: P-beta-1 score
Men: aged 24-50										
Uncomplicated diabetes	11	37½	10	252 *(220-290)	8.7 (5-11)	1.1 (0-3)	75	40	239 (190-300)	1.2† (0-5)
With diabetic retinitis	4	39	18	310 (230-390)	15.8 (15-17)	4.1 (3-6)	—	—	—	—
Men: aged over 50										
Uncomplicated diabetes	15	60	7	320 (210-440)	12.5 (8-19)	1.6 (0-4)	138	64	255 (220-310)	1.5† (0-5)
With coronary artery disease (three active)	9	62	11	275 (190-350)	12.7 (6-31)	2.3 (0-4)	28	58½	290 (220-405)	3.6† (1.5-6)
With peripheral arteriosclerosis	6	69½	18	273 (230-310)	11.5 (7-15)	1.3 (0.5-2.5)	25	65	236 (150-300)	1.8 (0-4)
With diabetic retinitis	8	64	16½	316 (240-350)	15.5 (10-31)	1.9 (0-4.5)	—	—	—	—
Women: all ages (not pregnant)										
Uncomplicated diabetes	9	42½	8½	304 (160-440)	11.9 (4-15)	2.1 (0.5-5)	19	41½	256 (190-370)	1.0 (0-4)
With coronary artery disease (none active)	4	66½	14½	330 (240-470)	14.5 (10-19)	1.6 (0-4.5)	11	60	350 (280-530)	3.3 (0-7)
With peripheral arteriosclerosis	6	59	19	315 (290-350)	10.6 (7-20)	1.4 (0.5-3.5)	4	62	290 (220-330)	1.9 (0-6)
With diabetic retinitis	15	54	19	300 (260-350)	10.0 (6-20)	1.5 (0-3.5)	—	—	—	—

*Numbers in parentheses give the range of values.
†Data previously published (Weitzman & Smith[28])

different weeks of pregnancy are set out in table 2. In normal women the cholesterol rises steadily, then falls soon after delivery. The p-β-l shows no increase before the thirtieth week; there is then a modest increase and it returns to normal shortly after delivery. The results in the diabetic group are different; in the successful pregnancies the cholesterol is initially higher and remains almost constant throughout; the p-β-l increases after the twenty-fifth week and is considerably elevated at term, but again falls rapidly after delivery. In the unsuccessful pregnancies the p-β-l is slightly raised before the twenty-fifth week, and increases to a very high level in the first two weeks postpartum; these changes are illustrated in figure 2. Figures 3 and 4 are photographs of the changing lipid patterns in two patients.

The control of diabetes during pregnancy in both the successful and unsuccessful groups of women was good in two thirds and fair in one third. Control was poor in only one subject and her pregnancy was successful.

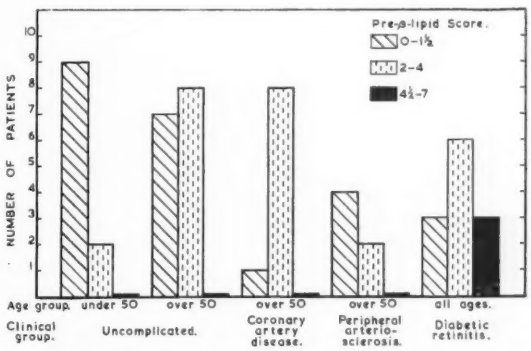


FIG. 1. The number of patients with minimal (0-1½), moderate (2-4), and large (4½-7) pre-β-lipid scores in different groups of diabetic men.

DISCUSSION

The aim of this study was to answer the following questions:

(1) As the p-β-l band is increased in patients suffering from active coronary artery disease and myocardial infarction, does it increase before the acute

TABLE 2

Cholesterol and pre-β-lipid during pregnancy and puerperium in normal and diabetic women

			Less than 25th week	25th-30th week	31st-35th week (31st week—delivery for diabetics)†	36th week —delivery	1-2 weeks p.p.	More than 2 weeks p.p.
NORMAL PREGNANCIES	P-β-1	Average	1.7 (10)*	1.7 (10)	2.5 (11)	2.3 (11)	1.1 (10)	—
		Range	½-3½	½-4	1-4½	1-4	½-2	
	Cholesterol mg./100 ml.	Average	229 (10)	232 (10)	300 (11)	319 (10)	236 (10)	—
		Range	166-290	135-310	201-400	250-430	180-300	
SUCCESSFUL DIABETIC PREGNANCIES	P-β-1	Average	1.6 (14)	2.7 (15)	3.6 (26)	—	2.7 (13)	1.8 (11)
		Range	0-5	0-6	2½-6		0-5½	0-4
	Cholesterol mg./100 ml.	Average	271 (8)	277 (11)	287 (22)	—	279 (12)	272 (11)
		Range	235-315	218-390	219-385		225-359	237-302
UNSUCCESSFUL DIABETIC PREGNANCIES	P-β-1	Average	2.1 (4)	4.0 (3)	4.4 (7)	—	5.3 (4)	2.8 (8)
		Range	½-4	3½-4½	2-6		5-6	0-5
	Cholesterol mg./100 ml.	Average	227 (4)	298 (3)	287 (6)	—	256 (4)	302 (8)
		Range	210-243	250-334	255-325	—	200-320	238-370

*Number of patients in parentheses.
†The third period is for the thirty-first to thirty-fifth weeks for normals, and the thirty-first week to delivery (generally in the thirty-sixth week) for diabetics.

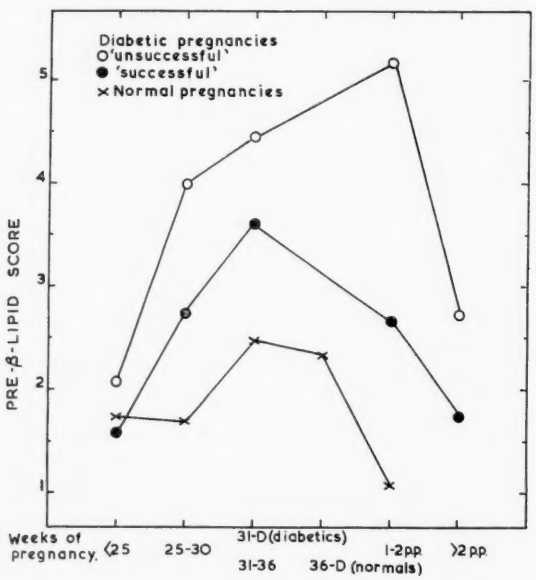


FIG. 2. The average pre-β-lipid score in successive periods of pregnancy and puerperium.
In the diabetics the third point refers to the period thirty-first week to delivery (generally in the thirty-sixth week); in the normal women the fourth point refers to the period thirty-sixth week to delivery.

clinical manifestations?

(2) Does the diabetic state, which apparently favors the development of degenerative vascular changes, in itself influence the production of p-β-l?

The answer appears to be "no" to both questions except possibly in pregnancy where production of a large p-β-l band may be associated with the diabetic state. If it is correct to think that diabetic men are more prone than normal men to atherosclerosis and coronary artery disease this study suggests that an increased p-β-l does not precede the vascular lesions and thus does not necessarily take part in their pathogenesis.

The average p-β-l score for all diabetics studied here is about the same as for comparable nondiabetic controls. Several authors (Harris et al.;[19] Tuller et al.;[20] Dangerfield and Smith[3]) have shown abnormal lipids during diabetic ketosis, but although not all our patients were well controlled none was examined during a ketotic period. An increase in p-β-l was associated with diabetic retinopathy in the younger men, but not in the older men or the women. This probably reflects the fact that in the four young men the retinitis was severe and rapidly progressive, and two of them also showed signs of nephropathy, whereas in the older men it was stable or only slowly progressive. A greater degree of lipid abnormality in young patients has also

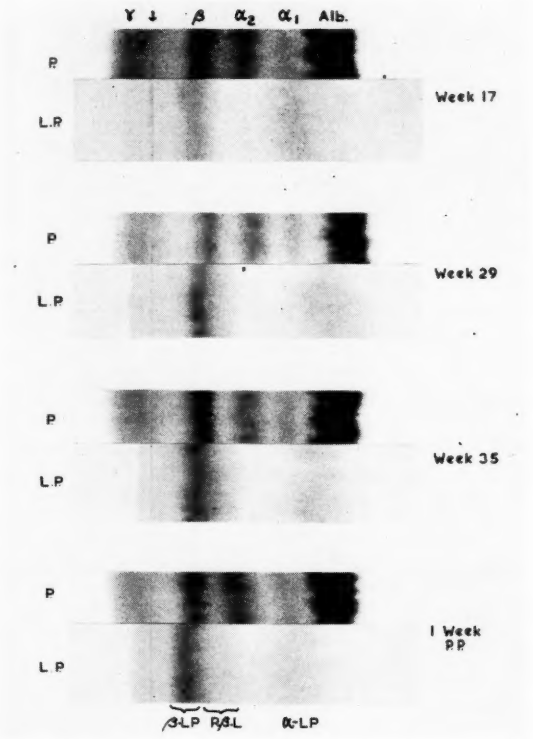


FIG. 3. Lipoprotein patterns at different stages of a successful diabetic pregnancy. A normal infant was born at thirty-seven weeks.
P. Protein pattern.
L.P. Lipoprotein pattern.
↓ Point of application of serum.

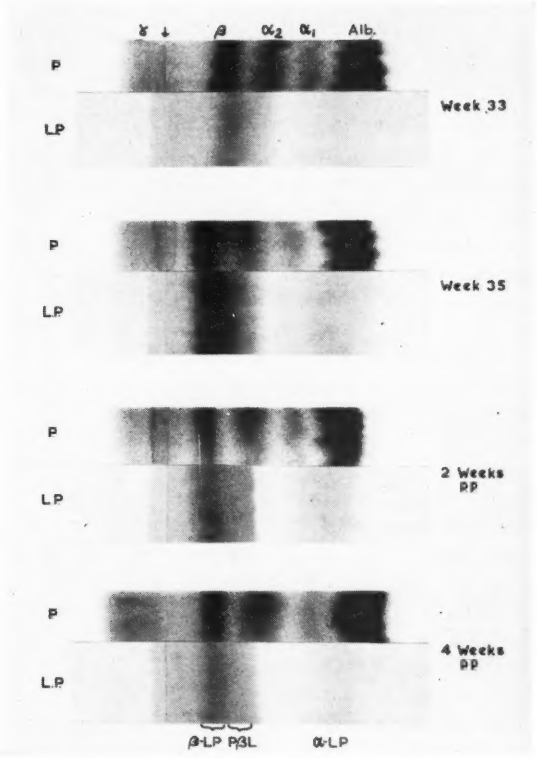


FIG. 4. Lipoprotein patterns in the later stages of an unsuccessful diabetic pregnancy. A stillborn infant was produced at thirty-seven weeks. Note the gross lipid abnormality at thirty-five weeks and the persistence of a strong pre-β-lipid band four weeks postpartum.
P. Protein pattern.
L.P. Lipoprotein pattern.
↓ Point of application of serum.

been observed in coronary artery disease.[5,8,9]

In normal pregnant women a small increase in the p-β-l begins about the thirty-first week and seems to parallel the increase in cholesterol which has already been reported (Oliver and Boyd;[21] Ditzel and Moinat[22]); the cholesterol and p-β-l drop sharply after the birth. In diabetic pregnant women there is a considerable increase in p-β-l starting earlier than in normals and before the twenty-fifth week in the unsuccessful cases. In these latter pregnancies the p-β-l remains grossly elevated during the first two weeks postpartum, but this abnormality is not associated with poor diabetic control. In the predelivery period the overlap between individual successful and unsuccessful cases makes it doubtful if determination of the p-β-l will provide much help in predicting the outcome of a diabetic pregnancy.

The factors involved in this large production of p-β-l are unknown, but it seems clear from this study that these abnormal lipoproteins are not exclusively associated with atherosclerotic vascular disease.

SUMMARY

An abnormal serum lipoprotein component, the pre-β-lipid (p-β-l), is known to occur in patients with clinical coronary artery disease, but it is not clear whether it arises as a result of myocardial ischemia or precedes it and contributes to the pathogenesis of atherosclerosis. This component was therefore studied in seventy-six diabetics of several years' standing on the assumption that they would have more atherosclerosis than normals of the same age.

There was no difference between patients with uncomplicated diabetes and normal controls in the amount or frequency of occurrence of the p-β-l. This strongly suggests that this lipoprotein is not implicated in the

development of cardiovascular disease in diabetics.

In pregnant diabetic women there was a considerable increase in p-β-l at term and in the first two weeks postpartum. In the patients in which the pregnancy was unsuccessful the p-β-l became grossly elevated.

SUMMARIO IN INTERLINGUA

Configurationes de Lipoproteina in Diabete, e le Alterationes Occurrente Durante le Pregnantia

Es cognoscite que un anormal componente de lipoproteina seral, i.e. le lipido pre-β (*p*-β-l), occurre in patientes con morbo de arteria coronari, sed il non es clar si illo resulta del ischemia myocardial o si illo pre-existe e contribue al pathogenese de atherosclerosis. Pro iste rationes, le mentionate componente esseva studiate in septanta-sex diabeticos con anamneses diabetic de plure annos de duration, a base del supposition que iste patientes deberea haber plus atherosclerosis que subjectos normal del mesme gruppos de etate.

Esseva trovate nulle differentia inter patientes con non complicate diabete e normal subjectos de controlo con respecto al quantitate o al frequentia del occurrentia de p-β-l. Isto suggere fortemente que le lipoproteina in question non es implicate in le disveloppamento de morbo cardiovascular in diabeticos.

In gravidas diabetic esseva trovate un augmento considerabile del concentration de p-β-l al tempore del parturition e durante le duo prime septimanas post parto. In le patientes in qui le pregnantia non esseva successose, le nivello de p-β-l deveniva grossiermente elevate.

ACKNOWLEDGMENT

We wish to thank the British Council for awarding a scholarship to Dr. Vernet (present address: Policlinique Universitaire de Medecine, Geneva, Switzerland), the Medical Research Council for financial support for Dr. Smith, and Dr. J. D. N. Nabarro for his help, criticism and advice. We would also like to thank Dr. W. G. Oakley, Diabetic Clinic, King's College Hospital, London, and Professor D. M. Dunlop, Department of Therapeutics, Royal Infirmary, Edinburgh, who very kindly permitted us to study their pregnant diabetic patients; also the laboratory staff of the Institute of Clinical Research and Miss P. A. Wilkinson of the Courtauld Institute of Biochemistry for technical assistance and serum cholesterol estimations.

REFERENCES

[1] Oliver, M. F., and Boyd, G. B.: Serum lipoprotein patterns in coronary sclerosis and associated conditions. Brit. Heart J. *17*:299, 1955.

[2] Lindgren, F. T., and Gofman, J. W.: The role of lipoproteins in coronary disease. Bull. Swiss Acad. med. Sci. *13*: 152, 1957.

[3] Dangerfield, W. G., and Smith, E. B.: An investigation of serum lipids and lipoproteins by paper electrophoresis. J. Clin. Path. *8*:132, 1955.

[4] Besterman, E. M. M.: Lipoproteins in coronary artery disease. Brit. Heart J. *19*:503, 1957.

[5] Smith, E. B.: Lipoprotein patterns in myocardial infarction. Lancet *2*:910, 1957.

[6] Dodds, E. C., and Mills, G. L.: Influence of myocardial infarction on plasma lipoprotein concentration. Lancet *1*: 1160, 1959.

[7] Cooperative study of lipoproteins and atherosclerosis: Evaluation of serum lipoprotein and cholesterol measurements as predictors of clinical complications of atherosclerosis. Circulation *14*:691, 1956.

[8] Lawry, E. Y., Mann, G. V., Peterson, A., Wysocki, A. P., O'Connell, R., and Stare, F. J.: Cholesterol and β-lipoprotein in the serum of Americans. Am. J. Med. *22*:603, 1957.

[9] Page, I. H., and Lewis, L. A.: Lipoproteins, cholesterol and serum proteins as predictors of myocardial infarction. Circulation *20*:1011, 1959.

[10] Lewis, J. G., and Symons, C.: Vascular disease in a diabetic clinic. Lancet *2*:985, 1958.

[11] Liebow, I. M., Hellerstein, H. K., and Miller, M.: Arteriosclerotic heart disease in diabetes mellitus. Am. J. Med. *18*: 438, 1955.

[12] Dawber, T. R., Moore, F. E., and Mann, G. V.: Coronary heart disease in the Framingham study. Am. J. Public Health *47*: Supplement to No. 4, 1957.

[13] Lisa, J. R., Magiday, M., Galloway, I., and Hart, J. F.: Arteriosclerosis with diabetes mellitus. J.A.M.A. *120*:192.

[14] Stearns, S., Schlesinger, M. J., and Rudy, A.: Incidence and clinical significance of coronary artery disease in diabetes mellitus. Arch. Int. Med. *80*:463, 1947.

[15] Bell, E. T.: A postmortem study of vascular disease in diabetics. Arch. Path. *53*:444, 1952.

[16] Bloor, W. R.: The determination of cholesterol in blood. J. Biol. Chem. *24*:227, 1916.

[17] Bernfeld, P., Berkowitz, M. E., and Donahue, V. M.: A simple nephelometric method for the determination of human serum β-lipoprotein. J. Clin. Investigation *36*:1363.

[18] Oncley, J. L., Walton, K. W., and Cornwell, D. G.: A rapid method for the bulk isolation of β-lipoprotein from human plasma. J. Am. Chem. Soc. *79*:4666, 1957.

[19] Harris, L. V. D., Albrink, M. J., van Eck, W. F., Mann, E. B., and Peters, J. P.: Serum lipids in diabetic acidosis. Metabolism *2*:120, 1953.

[20] Tuller, E. F., Mann, G. V., Schertenleib, F., Roehrig, C. B., and Root, H. F.: The effects of diabetic acidosis and coma on the serum lipoproteins and cholesterol. Diabetes *3*: 279, 1954.

[21] Oliver, M. F., and Boyd, G. B.: Plasma lipid and serum lipoprotein patterns during pregnancy and puerperium. Clin. Science *14*:15, 1955.

[22] Ditzel, J., and Moinat, P.: Changes in serum proteins, lipoproteins and protein-bound carbohydrates in relation to pathologic alterations in the microcirculation of diabetic subjects. J. Lab. & Clin. Med. *54*:843, 1959.

[23] Weitzman, D., and Smith, E. B.: Ischemic electrocardiograms in symptomless men with a note on the serum lipoproteins. Brit. M. J. *2*:162, 1959.

Calibration of a Simplified Cortisone Glucose Tolerance Test

C. R. Klimt, M.D., Dr.P.H., F. W. Wolff, M.D., C. Silverman, M.D., Dr.P.H., and J. Conant, B.Sc., Baltimore

The capacity of a person to dispose of ingested glucose is often referred to as his glucose tolerance, and glucose tolerance tests of various modifications have been used since the beginning of the century for the diagnosis of diabetes. Essentially they consist of glucose loading with 1 to 1.75 gm. per kilogram of body weight of a fasting person and the determination of blood glucose concentration at regular intervals thereafter, usually up to three hours. In a normal person a peak is reached between thirty and 120 minutes after glucose loading and after a negative phase, with a low point around the third hour, fasting glucose concentration is regained. In diabetics there may or may not be an elevated fasting glucose level, higher and broader peaks and a delayed negative phase. This test has been extensively studied in the past and can be considered one of the well established diagnostic tests in a physician's armamentarium.[1-4] The test is designed principally to demonstrate the degree of insulin activity but is also influenced by several other factors, such as the rate of glucose absorption from the gastrointestinal tract, the level and rate of gluconeogenesis from liver and muscle glycogen and the rate of peripheral glucose utilization.

Many hormones have been shown to have insulin antagonistic action and some can cause temporary or permanent diabetes. Glucagon, a polypeptide of the pancreas, augments blood glucose levels by increasing the rate of glycogenolysis in the liver and thus acts as antagonist to insulin. Of the many hypophyseal hormones ACTH,[5] somatotropin and luteotropin have a demonstrable diabetogenic effect. Hydrocortisone and cortisone were found to produce a reversible diabetic state, as judged by the development of abnormal glucose tolerance values, in man after prolonged medication.[6]

In 1954 Fajans and Conn developed a test combining the demonstrated insulin antagonistic action of cortisone with the glucose tolerance test, hoping that the added stress provided by cortisone-induced gluconeogenesis would bring out cases with a relative deficiency of insulin, who would be more prone than others to develop frank diabetes in future.[7,8] The same authors reported in 1958 and 1959 on the results of follow-up studies on nondiabetic persons who had taken the cortisone glucose tolerance test in 1954. Among relatives of diabetics there had developed frank cases of diabetes in six out of thirty-four, whose cortisone test had been called positive, and in only one out of fifty-seven whose cortisone glucose tolerance test (CGTT) had been classified as negative. From the published accounts no concept of the range of the test in the various groups studied can be gained as only the means are given. Thus it is not apparent how much overlap occurred and whether or not bimodality in the frequency distribution of CGTT results had been observed. It appears that there was no firm ground for deciding on the dividing line between a normal and an abnormal CGTT, which is given as 160, 150 and 140 for the one, one and one-half, and two-hour test periods.[7,10]

The test, admittedly still insufficiently tried and geared to a cumbersome clinical procedure, was nevertheless considered the best one available for the prediction of prediabetes in man and thus to serve our ultimate study purpose, namely, to investigate familial aggregation and genetic patterns of prediabetes in a controlled field study.

OBJECTIVES AND DESIGN OF THE CALIBRATION STUDY

The purposes of the study were primarily to familiarize ourselves with the cortisone glucose tolerance test, to introduce and to try modifications intended to make the test practical for field use and to determine the characteristics of the distribution of blood glucose levels in presumed normals of different age, sex and racial characteristics, and to compare them with test results in

Presented at the Symposium on Genetic Aspects of Diabetes Mellitus and Prediabetes sponsored by The Clinical Society of the New York Diabetes Association, Inc., on Nov. 11, 1960.

From The Johns Hopkins University, Baltimore, Maryland. Dr. Klimt is now Associate Professor of Epidemiology, School of Public Health, University of Minnesota, Minneapolis, Minnesota.

known diabetics. It was found necessary to study the repeatability of the test in the same person, the correlation of the cortisone glucose tolerance test with the standard glucose tolerance test and the correlation of the different test periods within the cortisone and the glucose tolerance tests. The effect of a single oral cortisone dose one and one-half hours before glucose loading had to be demonstrated and the relationship of capillary to venous blood glucose levels established under the influence of cortisone. Lastly, the laboratory method had to be investigated for occurrence of systematic errors and for statistical parameters of chance errors.

It was thought that by studying frequency distributions of test results obtained from a group of "normal" persons, the occurrence of bimodality might possibly be observed and thus an objective dividing line between negative and positive test results determined. It was hoped that further insight into the meaning of the test would be gained from studying the area of overlap between presumed "normals" and known diabetics.

Once the CGTT technic had been simplifid and after we had become sufficiently versed in administering the modified test, we began the calibration study among volunteer inmates of Maryland's Houses of Correction. We aimed at testing approximately equal numbers from both sexes in three age groups, under 30, 30 to 49, and 50 and over, and to subdivide them equally into whites and nonwhites. When the tests were completed among the male inmates, every other volunteer was recalled for a repeat test and after that, those who had not been discharged in the meantime were subjected to an identical test except for the omission of cortisone. At the same time, one of us (F.W.W.) administered the CGTT to known diabetics at The Johns Hopkins Hospital. They were required to stop taking hypoglycemic treatment for the three days preceding the test. A group of female inmates, comparable to the male group with respect to age, sex and race was tested and a subsample submitted to the trying experience of five simultaneous capillary and venous bleedings in the course of one morning.

In the course of the study 2,206 blood glucose determinations were performed on 136 volunteers: seventeen clinic patients, twelve students and faculty members of The Johns Hopkins School of Hygiene, two officers of the female house of correction and 105 inmates. Three previously undiagnosed mild cases of diabetes were discovered among the 105 prison inmates, who had volunteered. It may be added parenthetically that, due to the disruption of family bonds which we found to be characteristic in particular of male prisoners, no reliable family histories could be obtained from prison inmates.

MATERIALS AND METHODS

Modifications of the original CGTT

A test suitable for the study of familial aggregation of prediabetes had to be both effective and practical. The procedure had to be simple enough to be used in the field, acceptable enough to apparently healthy persons so as not to be rejected out of hand, and painless enough so that children, in particular, would not be prompted to leave while the test was in progress. The cortisone glucose tolerance test developed by Conn and Fajans was considered the best studied test available. The clinical procedure used by the originators was, however, too cumbersome for field use. Apart from certain dietary rules for the three days preceding the actual test, it was required that the patient be given cortisone on the evening before the test and again two hours before drinking glucose in the morning. The test itself consisted of seven blood samples from the cubital vein taken in the course of three hours.

As a first measure to simplify the test to make it practical for a large-scale field study, it was decided to minimize dietary requirements and merely avoid carbohydrate starvation by requiring that bread or an equivalent source of starches be consumed with every one of three meals during the three days preceding the test. This requirement was easily met during the study conducted among prison inmates. This procedure, according to the subsequent publication of Dr. Wilkerson et al.,[4] should have been sufficient to prevent false-positive reactions to the glucose tolerance test due to carbohydrate starvation.

Second, it was felt appropriate to give the entire cortisone dose on the morning of the test, one and one-half hours before loading the patient with glucose. This decision was reached after we learned that maximal cortisone action could be expected to occur between three and four hours after ingestion.*

Third, it was considered imperative to reduce the number of blood samples. Omitting the half-hour testing periods at 30, 90 and 150 minutes after glucose loading left four test periods: fasting, 60, 120 and 180 minutes. During the calibration study in adult human volunteers a fasting, precortisone sampling period one and one-half hours before glucose loading was included, in order to follow cortisone action more completely.

Fourth, it was found that capillary blood specimens could be obtained easier, quicker and with less inconvenience to the patient, than venous ones. After some initial experimentation with the ear lobe, the fingertip

*From Dr. C. J. Migeon of The Johns Hopkins Department of Pediatric Endocrinology.

was found to be the better choice. Disposable lancets (Becton, Dickinson and Company) packed in tinfoil were used throughout which made possible the obtaining of two 0.1-ml. blood samples in immediate succession. In the beginning attempts were made to keep the prick open for later blood samples by applying a cotton ball soaked in heparin to the fingertip and keeping it in place with a rubber fingercot. This method did not prove too successful and was abandoned in favor of a separate prick on a different finger at each of the testing periods.

Fifth, the taking of capillary blood required the use of a micromethod for blood glucose determination. The "Glucostat" micromethod,* which yields true glucose values, has been used throughout.

Finally, it was thought that the methods for determining the glucose and cortisone doses could be improved, by making them dependent on a single physiological measurement. The scale chosen was body surface as determined from a Dubois nomogram, based on actual height and weight measurements. It was thought that 30 gm. of glucose per square meter of body surface would best be related to the insulin requirements of that person. The dose of cortisone selected was 50 mg. per square meter, or 100 mg. for an average size adult male. This would be about four times the daily production (25 mg.) of cortisone. This sliding dose scale permitted us in later studies to extend the use of the test to children, subjecting them to a stress comparable to the one given adults.

Figure 1 shows a modified Dubois nomogram which was used to determine glucose and cortisone dosage by connecting the height and weight scales by a straight line and reading off the respective dose levels at the intersect with the central scale. Glucose dose was determined to the nearest gram and cortisone to the nearest 5 mg., the latter being necessitated by the commercial availability of 25 mg. and 5 mg. tablets.

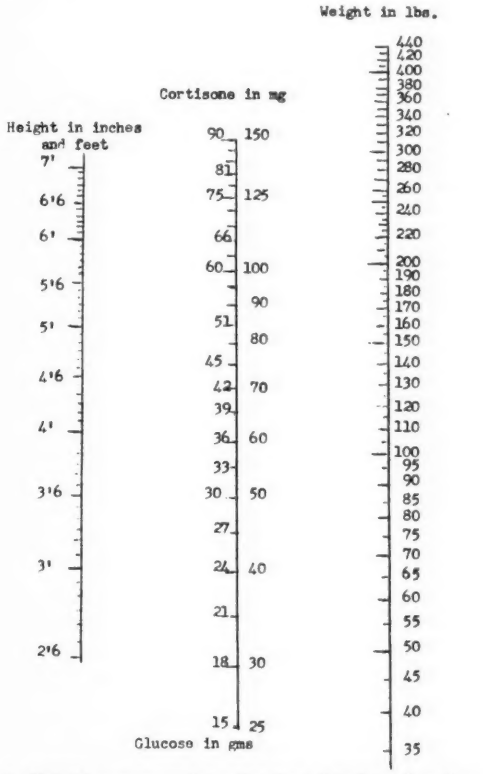


FIG. 1. Dubois' nomogram for estimation of body surface from height and weight measurements, adapted to permit determination of cortisone and glucose dose (30 gm. of glucose and 50 mg. of cortisone per square meter body surface).

RESULTS

1. *The frequency distribution of blood glucose values in 116 nondiabetic volunteers during the cortisone glucose tolerance test*

After exclusion of three volunteers with fasting blood glucose values in excess of 120 mg. per 100 ml., 116 (sixty-four males and fifty-two females) remained. These may then be considered an unselected population group, except for the fact that frank diabetics have been excluded. In the case of thirty-one male volunteers, where the cortisone test had been repeated on two or in a few instances more occasions, the average glucose levels were included in the frequency distribution.

Table 1 summarizes the results. The blood glucose values were grouped in intervals of 10 mg. per 100 ml.; for example, from 59.5 to 69.5 with a midpoint of 64.5 mg. per 100 ml. Statistical parameters were computed by using these midpoints rather than the actual values, as it could be expected that any asymmetry within the interval would be balanced by one in the opposite direction on the other half of the unimodal frequency distributions.

The overnight fasting values are shown in figure 2. A unimodal, nearly symmetrical distribution ranging from 59.5 to 119.5 mg. per 100 ml., with a mean of 87.9 is observed. The standard deviation is 9.8 mg. per 100 ml. and the standard error of the mean 0.9 milligram per 100 ml. At this stage of the test cortisone acetate was given by mouth in a dose of 50 mg. per square meter of body surface.

*Reagents and detailed description of the test available from Worthington Biochemical Corporation, Freehold, New Jersey.

TABLE 1

Frequency distribution of blood glucose levels during cortisone glucose tolerance test in 116 nondiabetic volunteers

Blood glucose in mg. per 100 ml.	F	0	1	2	3
60-69	4	1			13
70-79	12	15		4	24
80-89	55	50	1	12	25
90-99	33	34		11	22
100-109	9	14	1	16	14
110-119	3	2	1	19	11
120-129			11	7	2
130-139			9	12	2
140-149			10	6	2
150-159			8	9	1
160-169			14	7	
170-179			12	3	
180-189			11	2	
190-199			6	3	
200-209			9	2	
210-219			3	1	
220-229			8	1	
230-239			5		
240-249			3		
250-259					
260-269			2	1	
270-279			2		
$\bar{x}$	87.9	88.9	176.1	127.4	90.5
S.D.$_{\bar{x}}$	9.8	9.5	39.3	36.1	19.0
S.E.$_{\bar{x}}$	0.9	0.9	3.6	3.4	1.8

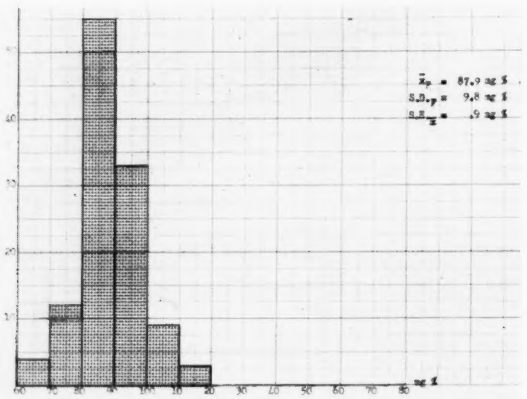


FIG. 2. Cortisone glucose tolerance test: frequency distribution of fasting blood glucose levels in 116 nondiabetic volunteers. $\bar{x}_F$ = 87.9 mg. %; S.D.$_F$ = 9.8 mg. %; S.E.$_{\bar{x}}$ = .9 mg. %.

One and one-half hours after cortisone ingestion we find a similar, if somewhat flatter, unimodal distribution (figure 3). The parameters are 88.9 mg. per 100 ml. for the mean, with an unchanged range from 59.5 to 119.5, a standard deviation of 9.5 mg. per 100 ml. and a standard error of the mean of 0.9 mg. per 100 ml. At this stage of the test the patient takes glucose in water flavored with citric acid in a dose of 30 gm. per square

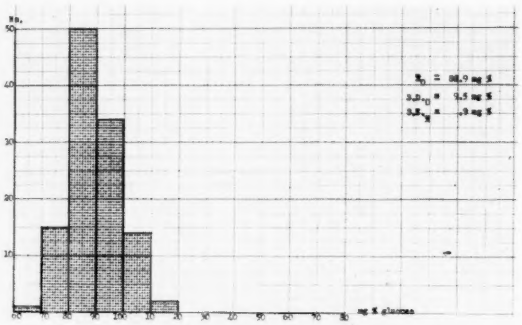


FIG. 3. One and one-half hours after oral cortisone acetate. $\bar{x}_O$ = 88.9 mg. %; S.D.$_O$ = 9.5 mg. %; S.E.$_{\bar{x}}$ = .9 mg. %.

meter of body surface.

One hour after glucose ingestion and two and one-half hours after cortisone by mouth, the distribution is still unimodal but there is a suggestion of positive skewness (figure 4). The range has increased from sixty at fasting stages to 200 mg. per 100 ml. (80-280 mg. per 100 ml.) with a mean blood glucose value of 176.1 mg. per 100 ml. This is reflected by a standard deviation of 39.3 mg. per 100 ml. and a standard error of 3.6 mg. per 100 ml.

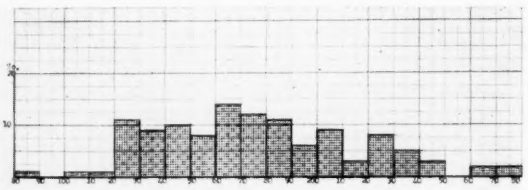


FIG. 4. One hour after glucose per os and two and one-half hours after cortisone acetate. $\bar{x}_1$ = 176.1 mg. %; S.D.$_1$ = 39.3 mg. %; S.E.$_{\bar{x}}$ = 3.6 mg. %.

Two hours after glucose and three and one-half hours after cortisone the distribution is still unimodal with an increased suggestion of positive skewness (figure 5). The range is still 69.5 to 269.5 mg. per 100 ml., but the mean has dropped to 127.4 mg. per 100 ml. The S.D. with 36.1 mg. per 100 ml. and the S.E. of the mean with 3.4 mg. per 100 ml. are essentially unchanged from the preceding level.

Three hours after glucose and four and one-half hours after oral cortisone the distribution is unimodal and positively skewed with a range from 59.5 to 159.5 (figure 6). The mean has returned to near fasting values (90.5 mg. per 100 ml.), which is misleading, as the

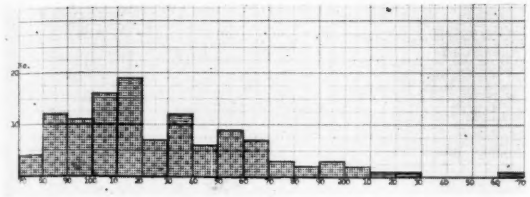


FIG. 5. Two hours after glucose per os and three and one-half hours after cortisone acetate. $\bar{x}_2$ = 127.4 mg. %; S.D.$_2$ = 36.1 mg. %; S.E.$_{\bar{x}}$ = 3.4 mg. %.

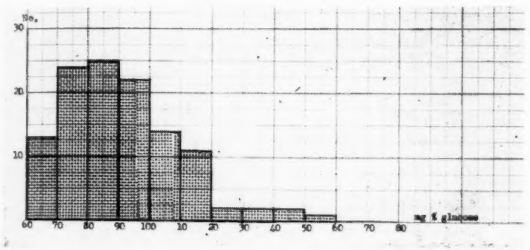


FIG. 6. Three hours after glucose per os and four and one-half hours after cortisone acetate. $\bar{x}_3$ = 90.5 mg. %; S.D.$_3$ = 19.0 mg. %; S.E.$_{\bar{x}}$ = 1.8 mg. %.

modal value is only about half in numbers of what it had been at fasting (twenty-five versus fifty-five). The S.D. is 19.0 and the S.E. of the mean 1.8. It appears that the greater frequency of glucose values below the mean, when compared with the fasting distribution, indicates that these persons had already reached a "negative phase" three hours after glucose ingestion, while others, notably those who still exceeded 120 mg. per 100 ml., were still on the decline. The latter group consisted of seven persons, or 6.6 per cent of the ones tested. Four of these seven were also among the upper 6 per cent of the values registered for the preceding time (two hours after glucose). None of them, however, was among the upper 6 per cent one hour after glucose although they were all well above the mean for the total group (196.4 versus 176.1).

It may be concluded that the absence of bimodality in the frequency distributions during the CGTT does not permit us to pinpoint a possibly "abnormal" or "prediabetic" population. The positive skewness observed for each of the last three test periods may, however, hide a second distribution so that when larger numbers are tested a second peak might become discernible at the upper end of the curve. From the study of Steinberg and Wilder,[11] who found 6.1 per cent diabetes in siblings of diabetics, and from the most complete case-finding program in an entire community done so far in Oxford, Massachusetts,[12] where 6.3 per cent of those aged sixty-five years and over were found to be diabetic, we may conclude that the maximal proportion of diabetes-prone persons would not exceed 6 per cent of an unselected group of people. Thus, at the most we may expect 6 per cent of the total area under the frequency distribution to form a second distinct distribution. This could only have been expected to become visible among 116 persons tested, if the two distributions had been completely separated.

2. *The frequency distribution of blood glucose values in forty-eight nondiabetic volunteers during the glucose tolerance test*

Forty-eight of the sixty-four nondiabetic male volunteers who had the CGTT also received a glucose tolerance test one to four weeks later, using identical procedures, i.e., overnight fasting, same glucose loading dose, same intervals for obtaining specimens, etc. The time of the day for obtaining fasting specimens was 8:30 a.m. for both the CGTT and the GTT. The GTT series took their glucose loading dose at this time, while the CGTT series took them one and one-half hours later. Thus the one-, two- and three-hour glucose determinations were, on the average, performed one and one-half hours earlier during the GTT. The twelve original volunteers not given the GTT had in the interval been discharged from the House of Correction, and might be assumed, on the average, not to differ with respect to their glucose tolerance from those who had been tested.

The GTT results are summarized in table 2 and illustrated in figures 7 to 10.

The fasting distribution is essentially unimodal (disregarding the irregularity of distribution due to small numbers between 89.5 and 119.5 mg. per 100 ml.), with a mean of 82 mg. per 100 ml.

The one hour after glucose loading distribution is unimodal and positively skewed, with a mean of 145.8 mg. per 100 ml. and a range from 99.5 to 219.5 mg. per 100 ml.

The two-hour distribution ranges from 49.5 to 169.5 mg. per 100 ml. with a mean of 105.1. It too is unimodal with positive skewness.

The three-hour distribution is strongly skewed to the right, ranges from 49.5 to 119.5 and has a mean of only 74.5 mg. per 100 ml., suggesting that here the majority, not under the influence of cortisone, have entered what has been termed a "negative phase."

The CGTT and the GTT results have been summarized in figures 11 and 12, respectively, where the means, plus and minus two standard errors of the mean and plus and minus two standard deviations are graphically

TABLE 2

Frequency distribution of blood glucose levels during glucose tolerance test in forty-eight nondiabetic male volunteers

Blood glucose in mg. per 100 ml.	0	1	2	3
50-59	1		1	3
60-69	1		2	18
70-79	22		4	15
80-89	17		8	5
90-99	1		7	3
100-109	4	3	8	3
110-119	2	7	4	1
120-129		8	4	
130-139		5	4	
140-149		4	4	
150-159		5	1	
160-169		5	1	
170-179		4		
180-189		4		
190-199		2		
200-209				
210-219		1		
$\bar{x}$	82.0	145.8	105.1	74.5
$S.D._x$	11.9	28.1	25.8	13.8
$S.E._{\bar{x}}$	1.7	4.1	3.7	2.0

FIG. 7. Glucose tolerance test: frequency distribution of fasting capillary blood glucose levels in forty-eight healthy volunteers. $\bar{x}_0 = 82$ mg. %; $S.D._0 = 11.94$ mg. %; $S.E._{\bar{x}} = 1.72$ mg. %.

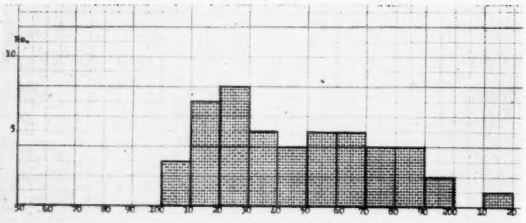


FIG. 8. One hour after oral glucose. $\bar{x}_1 = 145.75$ mg. %; $S.D._1 = 28.11$ mg. %; $S.E._{\bar{x}} = 4.06$ mg. %.

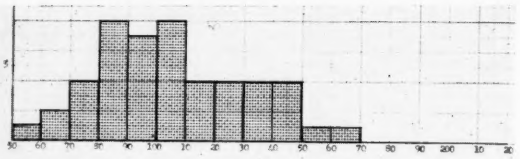


FIG. 9. Two hours after oral glucose. $\bar{x}_2 = 105.13$ mg. %; $S.D._2 = 25.80$ mg. %; $S.E._{\bar{x}} = 3.72$ mg. %.

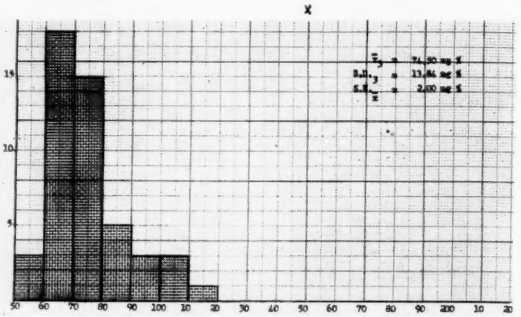


FIG. 10. Three hours after oral glucose. $\bar{x}_3 = 74.50$ mg. %; $S.D._3 = 13.84$ mg. %; $S.E._{\bar{x}} = 2.00$ mg. %.

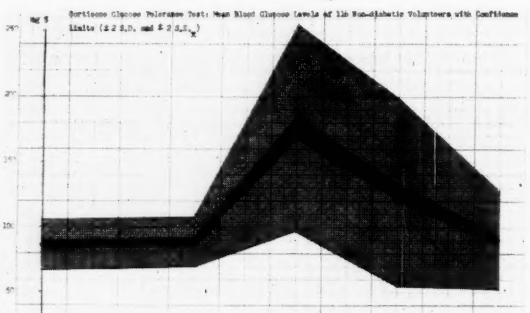


FIG. 11. Cortisone glucose tolerance test: mean blood glucose levels of 116 nondiabetic volunteers with confidence limits (±2 S.D. and ±2 S.E.$_{\bar{x}}$).

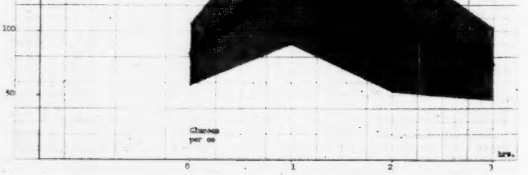


FIG. 12. Glucose tolerance test: mean blood glucose levels of forty-eight nondiabetic male volunteers with confidence limits (2 S.D. and 2 S.E.$_{\bar{x}}$).

shown. While the means appear equally stable during the CGTT and the GTT, the CGTT shows greater spread during the one-, two- and three-hour test levels. The mean levels of the CGTT all exceed the corresponding mean blood glucose values during the GTT. The difference at fasting was 5.9 mg. per 100 ml.; at one hour, 30.3; at two hours, 22.3; and at three hours 16.0 mg. per 100 ml.

In summary, we conclude that the frequency distribution of glucose values obtained during the GTT have the same unimodal and at times positively skewed appearance as has been observed for the CGTT. The latter, however, has a greater spread and higher means, particularly during the one-, two- and three-hour phase of the CGTT, when compared with the corresponding period during the GTT. A comparison of the two tests in the same individuals permitting the measurement of the cortisone effect will be presented separately.

3. *The cortisone glucose tolerance test in diabetics*

Twenty diabetics underwent the CGTT. The diagnosis of diabetes had been made independently in all but three cases, which were discovered during the study in the Maryland Houses of Correction. Diabetics, who were either on tolbutamide or on insulin treatment, were required to discontinue treatment for three days before taking the test.

Table 3 gives a summary of the results. Five out of twenty (25 per cent) would not have been diagnosed on the basis of fasting blood glucose levels in excess of 120 mg. per 100 ml. The mean fasting sugar value was, however, 181 mg. per 100 ml., about twice that of the nondiabetic group. The zero-, one-, two- and three-hour means were 177.5, 320.9, 302.5 and 251.3 mg. per 100 ml., respectively.

Considering the overlap of glucose levels in these diabetics with the 116 nondiabetic volunteers (table 1) one could at different test periods exclude varying proportions of nondiabetics from a suspicion of diabetes, as follows:

Test period	Proportion	Per cent	Lowest level (in mg. per 100 ml.) observed among diabetics
1. At fasting level	16/116	13.8	79.5
2. One and one-half hours after cortisone	16/116	13.8	79.5
3. One hour after glucose	93/116	80.2	199.5
4. Two hours after glucose	82/116	70.7	139.5
5. Three hours after glucose	62/116	53.4	89.5

TABLE 3

Cortisone glucose tolerance test results in twenty diabetics

Midpoint in mg. per 100 ml. glucose	F	0	1	2	3
74.5					
84.5	1	2			
94.5	1	1			1
104.5	2				1
114.5	1				
124.5	1	3			
134.5	1				
144.5	1	2		1	2
154.5	2	3			2
164.5	1			1	
174.5	1				
184.5					
194.5		3		1	
204.5	1			1	
214.5	1		1		
224.5		1	1	2	4
234.5			1		
244.5	1	2		1	1
254.5	2	1	1		
264.5	1		5	1	
274.5	1	1			1
284.5			1	1	
294.5				1	
304.5					
314.5			1		2
324.5			1		
334.5		1		2	1
344.5				1	2
354.5	1		1	1	
364.5			1	1	1
374.5			2	2	1
384.5					
394.5				1	
404.5			1		
414.5					
424.5					1
434.5					
444.5			1		
454.5					
464.5			2	2	
Mean	181.0	177.5	320.9	302.5	251.3

These findings support the contention of the U.S. Public Health Service that the effectiveness of a screening blood glucose determination would be lowest at the fasting period and highest one hour after glucose ingestion or after a meal.

If those "nondiabetics" were screened out, whose CGTT results were below the minimum levels observed among the twenty diabetics during all test periods of the CGTT, we would have excluded 106 out of 116 nondiabetics, or 91.4 per cent. The ten remaining suspicious cases include seven of the top ranking twelve CGTT results, when ranked in order of the sum total of all five glucose levels obtained during the CGTT.

4. *The repeatability of the cortisone glucose tolerance test (CGTT) in the same person*

The CGTT has been repeated in thirty-one male vol-

unteers between one and three weeks after the first test. Every other male volunteer in the House of Correction, who had once volunteered, was chosen for the repeat test.

In the cases where more than two tests had been performed on one person, the first two were chosen throughout for correlation purposes.

At fasting levels there was no significant correlation.

Correlation between tests in the same person was highly significant one hour ($r = .81$), two hours ($r = .62$) and three hours ($r = .71$) after glucose.

In summary, it can be stated that while correlation of blood glucose levels in the same person during the fasting stage and one and one-half hours after oral cortisone was not demonstrable, there was significant correlation at one, two and three hours after glucose loading. Individual variation over a short period of time can, however, be quite marked, so that a rigid line of demarcation between positive and negative test results on the basis of a single test does not appear justified.

It should be noted that the mean values for the first test series exceeded the ones for the second series at all five testing periods. This undoubtedly was more than a chance effect. The cause might have been a systematic laboratory error or possibly a reduced effectiveness of cortisone during the second test. This last hypothesis is somewhat improbable as the fasting means also differ in the same direction. The laboratory technic as well as the two technicians remained the same throughout. It could be that the prisoners being unaware of the strictness of our fasting requirements, had had sweetened coffee before the first test, whereas they were completely fasting for the second test.

Under the prevailing circumstances it has not been possible either to confirm or to refute this hypothesis through direct questioning.

5. *The correlation of the CGTT and the GTT*

Fifty-one male volunteers wre subjected both to the CGTT and the GTT, the GTT always following the CGTT at an interval of one to four weeks. The same glucose dose was given for both tests although at times small changes in weight were noted between the two test periods. It has already been mentioned that the glucose tolerance test periods one, two and three preceded the corresponding ones duing the CGTT by one and one-half hours in terms of time of the day. Thus the period of fasting was shorter throughout the last three test periods for the GTT.

At fasting there was an insignificant difference between the CGTT and the GTT series. Correlation was significant and more uniform at the one-, two- and three-hour after glucose ingestion periods, with correlation coefficients of .65, .56 and .73, respectively. At each of these time periods the CGTT mean blood sugar exceeded the one for the GTT. At one hour the difference was 31.6 mg. per 100 ml., an increase of 20.9 per cent over the GTT level; at two hours it was 17.1 mg. per 100 ml., an increase of 15.4 per cent; and at three hours it was 11.2 mg. per 100 ml., an increase of 14.3 per cent. Thus maximal cortisone action in terms both of absolute and relative glucose increase in the blood apparently occurred two and one-half hours after it had been taken by mouth. There was still significant, if decreasing, action four and one-quarter hours after the ingestion of cortisone.

In view of these findings it was considered unnecessary to split the cortisone dose and administer one half the evening preceding the test and the second half two hours before glucose loading which was the procedure originally prescribed. There is reason to be cautious in the interpretation of a single test, CGTT or GTT, in view of the apparent variability of the reaction in one person after a maximal interval of only four weeks. This seems true in spite of the helpful factor that the prisoners all had the same carbohydrate-rich and protein-poor diet throughout the testing period.

6. *The correlation of blood glucose values in the same person during successive periods of the CGTT and the GTT*

A series of fifty-one male volunteers, who had both the CGTT and the GTT, was analyzed for within-test correlation for successive test periods. Significant correlation at the 99 per cent probability level was demonstrated throughout. Thus both for the CGTT and the GTT there was significant correlation between the fasting and one hour after glucose periods, between the one- and two-hour periods and between the two- and three-hour periods. It should be noted that the fasting level already forecasts to some extent the magnitude of the reaction after glucose loading and that throughout both the CGTT and the GTT successive blood glucose levels are significantly correlated. It would therefore be a mistake to base optimal evaluation of the test solely on a selected part of the results, as the one-, one and one-half- and two-hour levels, which are used exclusively by the originators of the CGTT.

To this series of observations, regression lines have been fitted and the significance of the difference in slope of corresponding lines for the CGTT and the GTT tested. There are six corresponding pairs of slopes:

Regression line:	Test Periods	Slope CGTT	Slope GTT
mean x when y is given	(F/1)	1.24	1.14
mean y when x is given	(F/1)	.14	.28
mean x when y is given	(1/2)	.59	.50
mean y when x is given	(1/2)	.54	.70
mean x when y is given	(2/3)	1.14	1.12
mean y when x is given	(2/3)	.53	.54

No pair of slopes differs significantly. This we would interpret as meaning that the CGTT and the GTT are measuring the same qualitative carbohydrate metabolic factor and consequently the same pathology, only they do so on a different scale. The CGTT scale lies higher and has a greater range than the GTT scale. The CGTT therefore offers a greater opportunity for discerning abnormality, not because it differs qualitatively from the GTT but because of its wider scale, on which bimodality, if present, could be seen more clearly.

7. *The laboratory error of the "Glucostat" micromethod for determining true capillary blood glucose levels*

In the course of the CGTT volunteer study there have been 1,103 duplicate capillary blood glucose determinations performed. At each time period of the test, two successive samples of 0.1 ml. of capillary blood were drawn from the same fingertip. The laboratory work on the duplicate specimens was generally performed during one series of glucose determinations and the final step, the reading of the spectrophotometer, was done consecutively, fasting specimen one from patient one, fasting specimen two from patient one, etc. It is recognized that the reading arrangement has been such that complete independence cannot be claimed. The technician could have been influenced by knowledge of the preceding reading coming from a replicate blood specimen.

Figure 13 gives the frequency distribution of differences between first and second duplicate sample with due regard to the sign of the difference. If the first test was higher than the second, the difference was considered positive and recorded on the right half of the frequency diagram; if the second test exceeded in value the first test it was counted for the left half. Adjusted percentages are shown for the irregular intervals +5 to +1 and — 1 to —5 and for the interval less than +1 to less than —1, called zero. The distribution is very nearly symmetrical, indicating the absence of a systematic error; it is unimodal but not normal in shape. The tail ends on either side are far too long for a normal distribution. The mean error irrespective of direction is 5.11 mg. per 100 ml.; when the sign before the error term is considered, the mean is +0.35 mg. per 100 ml.,

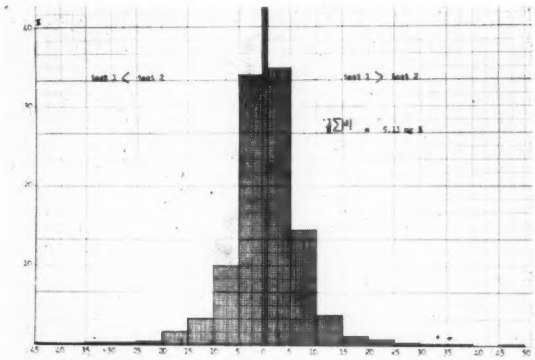


FIG. 13. Distribution of differences: 1,103 duplicate blood glucose determinations.

which does not differ significantly from zero.

It was not unexpected to find that the mean error without considering its sign increases with the height of the glucose level; while the mean errors with signs considered do not differ significantly from zero at the 99 per cent probability level.

mg. per 100 ml. glucose	Mean error	Mean error irrespective of sign	n
50-149	+ .14	4.46	853
150-249	+1.36	6.81	194
250+	— .02	9.32	54
Totals	+ .35	5.11	1,101

The difference between the mean errors irrespective of sign is significant at the 1 per cent level between 4.46 and 6.81, while the probability that the second difference between 6.81 and 9.32 is due to chance alone is about 4 per cent.

In figure 14 the mean absolute and relative errors are plotted by height of glucose level and least square fitted

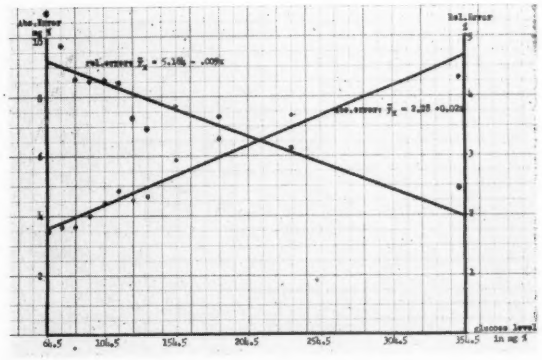


FIG. 14. Absolute and relative errors of 1,103 duplicate blood glucose determinations, grouped by height of glucose level, with fitted lines.

lines are shown. The absolute error increases in linear form at the rate of 2 mg. per cent for every 100 mg. per 100 ml. increase in glucose level, while the relative error (per cent error per glucose level interval of 10 mg. per 100 ml.) decreases also in linear form at the rate of 1.1 per cent points for every 100 mg. increase in glucose level. The absolute error increases between 64.5 mg. per 100 ml. glucose and 354.5, from 3.5 mg. per 100 ml. to 8.6 mg. per 100 ml. and the relative error decreases over the same range from 4.6 per cent to 2.0 per cent.

All specimens were precipitated immediately after they were obtained and separation of sediment and supernatant by centrifugation was performed on the same day. The test was completed either on the day of collection or up to a maximum of three days later. As the microtechnic has apparently as yet not been adapted to the use of sodium fluoride as a glucose preservative, storage of the supernatant was carried out at —20° C. Tests over a three-day period showed that the drop in glucose content averaged 2.9 mg. per 100 ml., which is well below the average laboratory error of 5.1 mg. per 100 ml. It is felt therefore that delays between sampling and completing the laboratory test did not add materially to the variability of the results.

8. *Correlation of simultaneously taken capillary and venous blood glucose values during the cortisone glucose tolerance test*

Thirteen female nondiabetic volunteers were given a CGTT as previously described, but were in addition also bled with a delay of less than two minutes from the cubital vein of the same arm from which the capillary specimens had been obtained from the fingertip. The venous blood was treated in identical fashion to the capillary blood: 0.1 ml. was pipetted from a test tube into 1.9 ml. of distilled water and immediately precipitated with 1.0 ml. each of $Ba(OH)_2$ and $ZnSo_4$. The thirteen volunteers were seen at the time of the test for the first time and were unselected except for the fact that they were part of a group of female prisoners who had volunteered for medical research, which involved taking of blood specimens.

Table 4 gives the individual blood glucose values, their means, variances and variances of the mean, the differences between the capillary and venous means and the results of t tests for the significance of these differences. For the fasting, zero and third hour the differences were insignificant; for the first hour the mean difference is 24 mg. per 100 ml. and for the second 13.3 mg. per 100 ml. With the numbers available, only the first-hour value is significantly different at the 95 per cent probability level.

Table 5 lists the differences by sign between capillary and venous blood glucose levels for the same thirteen individuals. Also given are their age, glucose and cortisone dose received, as well as the results in terms of statistical significance, when the sign test was used. In addition to the one-hour A/V difference, here also the two-hour A/V difference has statistical significance. All thirteen glucose values were higher in capillary

TABLE 4

Simultaneous capillary and venous blood glucose values during cortisone glucose tolerance test on thirteen female nondiabetic volunteers

	F		0		1		2		3	
Case No.	C	V	C	V	C	V	C	V	C	V
1	86	86	89	91	181	152	130	118	109	103
2	83	80	80	88	152	129	97	88	95	96
3	83	84	82	81	159	133	76	62	78	77
4	90	90	76	84	181	175	209	197	155	152
5	88	94	91	91	146	119	108	88	87	81
6	103	102	100	100	242	223	103	91	76	81
7	—	—	106	110	160	156	121	106	88	88
8	—	—	102	102	222	195	157	141	94	89
9	—	—	92	96	223	175	167	144	97	94
10	—	—	76	74	164	132	132	128	79	85
11	—	—	99	105	172	155	116	101	97	97
12	—	—	97	101	200	189	190	184	121	120
13	—	—	87	92	214	171	98	83	75	69
$\bar{x}$	88.8	89.3	90.5	93.5	185.8	161.8	131.1	117.8	96.2	94.8
v	50.61	45.13	99.77	98.27	972.6	901.8	1537.2	1602.0	490.0	460.0
$v_{\bar{x}}$	8.435	7.521	7.675	7.559	74.82	69.37	118.3	123.2	37.69	35.39
$\bar{d}$	+.5		+3.0		—24.0		—13.3		—1.4	
p	n.s.		n.s.		p=.05		n.s.		n.s.	

TABLE 5

Differences between capillary and venous blood glucose levels during cortisone glucose tolerance test on thirteen female nondiabetic volunteers

Case No.	F	0	1	2	3	Cortisone dose in mg.	Glucose dose in gm.	Age
1	0	+2	—29	—12	—6	85	51	28
2	—3	+8	—23	— 9	+1	75	46	19
3	+1	—1	—26	—14	—1	85	51	30
4	0	+8	— 6	—12	—3	85	52	22
5	+6	0	—27	—20	—6	100	58	42
6	—1	0	—19	—12	+5	75	47	25
7	—	+4	— 4	—15	0	80	50	20
8	—	0	—27	—16	—5	80	48	20
9	—	+4	—48	—23	—3	70	43	22
10	—	—2	—32	— 4	+6	80	51	22
11	—	+6	—17	—15	0	90	55	30
12	—	+4	—11	— 6	—1	90	54	47
13	—	+5	—43	—15	—6	90	53	32
mean	+ .5	+2.9	—24.0	—13.3	—1.5	83.5	50.7	27.6
max. neg. diff.	—3	—2	—48	—23	—6			
range	—3/+6	—2/+8	—48/—4	—23/—4	—6/+6	70/100	43/58	19/47
number with neg. diff. over total with a diff.	2/4	2/10	0/13	0/13	3/11			
p (one-sided sign test)	.5	.055	<.000	<.000	.113			

than in venous blood at each of these two time levels.

9. *The effect of age, race and sex on the outcome of the CGTT and the normal GTT*

The results of the CGTT in 116 nondiabetic volunteers were tested for age, sex and race effects by the analysis of variance technic and by means of multiple *t* tests on standardized means and variances of the mean. The same technics were also used on forty-eight GTT results from male volunteers, which were analyzed for age and race effects.

The effect of *age,* applying the analysis of variance test, was significant both for the CGTT and the GTT series at the 5 per cent but not at the 1 per cent level. Test periods alone, as has already been pointed out, were highly correlated in both tests. The results of the analysis of variance tests have been summarized in table 6A and B. Significant interaction, a concept which is not too readily understood, may be defined as a diagonal effect. In this case an effect of the test periods on age, in excess of the pure test period and age correlations, which could be termed vertical and horizontal, from the position they take on an analysis of variance table. The second method mentioned, i.e., multiple *t* tests on differences between standardized means was used in an effort to determine the specific values which differ from the null hypothesis. The results have been summarized in table 7A and B. For the CGTT the fasting, zero, two- and three-hour values are not significantly different between age group two (thirty to forty-nine years) and age group three (fifty years and above), while the one-hour value is significantly different at the 1 per cent level. The magnitude of the two-hour difference and the similarity of sign (—20 mg. per cent) makes it probable that this difference, too, is meaningful in spite of the absence of statistical significance. There are no consistent differences between the youngest age group (seventeen to twenty-nine years) and the middle age group (thirty to thirty-nine years). The comparison of sex and race standardized means for age differences in the GTT has yielded no specific significantly different values, although there is a difference of —15.6 mg. per cent at the one-hour period between age groups one and two, and a difference of —23.8 mg. per cent at the two-hour period between age groups two and three.

It may be concluded that a pure age effect has been shown to exist for the CGTT at the one-hour test period, where the group aged fifty and over exceeds the preceding age group, on the average, by 26.5 mg. per 100 ml. The difference of 20.1 mg. per 100 ml. between the means of the same age groups at the two-hour test period is probably also meaningful. There appears to be no age effect on the three "fasting" periods (F, 0 and 3). No age effect is seen when ages seventeen to twenty-nine are compared with those aged thirty to forty-nine.

In the GTT series of forty-eight, no significant age effect has been demonstrated for any specific test period, although an over-all age effect has been shown to exist.

The evidence for the existence of race differences is

contradictory and in a biological sense difficult to explain. In the CGTT series there is no significant race effect. There is significant interaction between the test period and race. In the GTT series the probability of a chance effect to explain observed race differences lies between 1 and 5 per cent. The probability of significant interaction lies equally between 1 and 5 per cent. (See table 6C and D.)

TABLE 6

Summary of analysis of variance test results for effects of age, race, sex, and within test periods as well as their interactions

A. Age, test-periods and interaction during CGTT:			
Age	F(2,565) =	4.112	p between 1% - 5%
Test-period	F(4,565) =	259.271	p less than 1%
Interaction	F(8,565) =	3.187	p less than 1%
B. Age, test-periods and interaction during GTT:			
Age	F(2,180) =	5.277	p less than 1%
Test-period	F(3,180) =	121.980	p less than 1%
Interaction	F(6,180) =	2.938	p less than 1%
C. Race, test-periods and interaction during CGTT:			
Race	F(1,570) =	.382	p not significant
Test-period	F(4,570) =	253.441	p less than 1%
Interaction	F(4,570) =	3.768	p less than 1%
D. Race, test-periods and interaction during GTT:			
Race	F(1,184) =	5.795	p between 1% - 5%
Test-period	F(3,184) =	114.605	p less than 1%
Interaction	F(3,184) =	3.509	p between 1% - 5%
E. Sex, test-periods and interaction during CGTT:			
Sex	F(1,570) =	5.077	p between 1% - 5%
Test-period	F(4,570) =	251.555	p less than 1%
Interaction	F(4,570) =	1.561	p not significant

Looking at table 7C for a test-period specific comparison of age and sex standardized means for race effects in the CGTT series, we find that a sizeable difference exists at the one-hour test period only, where whites exceed nonwhites on the average by 18.0 mg. per 100 ml. A similar comparison in the GTT series (table 7D) shows a difference of similar magnitude in the same direction at the same one-hour test period, where whites exceed nonwhites on the average by 21.3 mg. per 100 ml. These differences are significant at the 1 per cent level.

The absence of differences of smaller size but equal direction at the two-hour test periods of the CGTT and the GTT makes it difficult to believe that a consistent and biologically meaningful race difference has been observed. It remains, therefore, only to restate the findings, i.e., that in the two test series whites exceeded nonwhites at the one-hour test period only. The magnitude of the average absolute differences was similar for the CGTT and the GTT in spite of the generally higher glucose levels during the CGTT when compared with the corresponding ones during the GTT.

The evidence for the existence of *sex* differences during the CGTT is quite definite. From the analysis of variance (table 6E) we learn that sex differences have a probability of only about 3 per cent to be due to chance alone. Interaction is nonsignificant.

A comparison of age and race standardized means for sex effect (see table 7E) shows that at all five test periods females exceed males in average blood glucose values. The differences are significant at the three fasting periods (F, 0 and 3) and are not statistically significant at the one- and two-hour periods, although of about the same order of magnitude, and direction. (With the number of observations being equal at all test periods, lack of significance is solely due to the greater spread of observations.)

It would be interesting to speculate whether these findings are due to a true greater frequency of carbohydrate metabolic disorders in an "unselected" group of females, supported by the near-universal findings of a greater frequency of diabetes in females. If this were not the case we would be required to assume a physiological difference between the sexes and to set up different diagnostic standards for them.

10. *Glycosuria and blood glucose levels*

During the entire study urine specimens were collected immediately after the taking of blood specimens. The volunteers were asked to empty the bladder each time a specimen was required and not to urinate between collection times. The urine samples were tested with Clinitest tablets and the results recorded according to color as negative, plus-minus, one, two, three and four plus. The principal purpose of performing the urine test was to correlate the urine findings with the ranked CGTT results in order to determine the accuracy of a simplified diagnostic test such as has been utilized in England by Professor Russell Fraser of the postgraduate medical school in Hammersmith, London (personal communication of Dr. David Rabinovic).

If we consider the upper 6 per cent of the summed and ranked CGTT results as our best estimate of the true positives (see previous reference to (11) and (12) for reasons for selecting 6 per cent), then the *sensitivity* of the urine test, e.g., a positive result (plus-minus or more) at any time during the cortisone test, would be:

$$\frac{\text{True positives called positive by test}}{\text{True positives}} = \frac{6}{7} = 85.7 \text{ per cent}$$

and the corresponding *specificity* defined as follows would be:

$$\frac{\text{True negatives called negative by test}}{\text{True negatives}} = \frac{93}{109} = 85.3 \text{ per cent}$$

TABLE 7

The effect of age, sex and race evaluated from a comparison of standardized mean blood glucose values during the CGTT and the GTT in nondiabetic volunteers

A. Age effect during CGTT:

CGTT time period	AG 1 17-29	Diff.	Signif.	AG 2 30-49	Diff.	Signif.	AG 3 50 over
F	88.1	—1.2	n.s.	89.3	+ 6.7	n.s.	82.6
0	89.6	—1.0	n.s.	90.6	+ 4.5	n.s.	86.1
1	172.7	+4.5	n.s.	168.2	—26.5	*sign.*	194.7
2	122.7	+2.8	n.s.	119.9	—20.1	n.s.	140.0
3	89.1	— .4	n.s.	89.5	— 2.2	n.s.	91.7
N	41			49			26

B. Age effect during GTT:

CGTT time period	AG 1	Diff.	Signif.	AG 2	Diff.	Signif.	AG 3
0	83.4	— .6	n.s.	84.0	+ 4.5	n.s.	79.5
1	133.9	—15.6	n.s.	149.5	— .6	n.s.	150.1
2	100.9	+ 3.9	n.s.	97.0	—23.8	n.s.	120.8
3	75.2	+ 4.0	n.s.	71.2	— 4.6	n.s.	75.8
N	17			18			13

C. Race effect during CGTT:

CGTT time period	White	Nonwhite	Diff.	Signif.
F	88.6	87.1	+ 1.5	n.s.
0	90.9	88.3	+ 3.8	n.s.
1	183.0	165.0	+18.0	*sign.*
2	124.2	130.1	— 5.9	n.s.
3	89.1	92.7	— 3.6	n.s.

D. Race effect during GTT:

GTT time period	White	Nonwhite	Diff.	Signif.
0	84.1	79.1	+ 5.0	n.s.
1	150.8	129.5	+21.3	*sign.*
2	104.6	105.3	— .8	n.s.
3	75.4	70.6	+ 4.8	n.s.
N	33	15		

E. Sex effect during CGTT:

CGTT time period	Male	Female	Diff.	Signif.
F	84.7	90.6	— 5.9	*sign.*
0	86.2	93.0	— 6.8	*sign.*
1	173.7	179.7	— 6.0	n.s.
2	124.2	127.1	— 2.9	n.s.
3	84.9	96.2	—11.3	*sign.*
N	64	52		

If the definition of a positive urine did not include plus-minus readings (which may be caused by errors in color perception or by nonglucose reducing substances) the *sensitivity* would become 4/7, or 57.1 per cent, and the *specificity* would be increased to 99/109, or 90.8 per cent.

DISCUSSION

In the absence of objective evidence, such as a bimodal frequency distribution would constitute, for determining prediabetics on the basis of the CGTT alone, there remain four avenues to follow:

1. To determine the upper 6 per cent of a population sample through a ranking procedure for the test. This has been done by summing all five CGTT test values for each person in the study and ranking them in order of magnitude. It so happens that the upper 6 per cent are also defined by summed values in excess of 700. Correlation of this ranking order with one determined by positive urines during the CGTT has been described.

2. To use a ranking method for the CGTT in first-degree relatives of diabetes cases and to compare their glucose levels with the ones in corresponding controls after standardization for age, sex and race. It could be that the frequency of high values among the relatives of diabetics would exceed the frequency with identical high values among the controls. This approach will be tried in a population-based field study.

3. To test a population-based sample of sufficient size capable of demonstrating the existence of a second distribution on the high side of the normal range, the area of which must not exceed 6 per cent of the total.

4. Follow-up over time of persons tested with the CGTT who constitute a probability sample of a defined population for the development of diabetes and correlating the predictive value of the test in terms of its original level, separately for relatives and nonrelatives of diabetics.

SUMMARY

The cortisone glucose tolerance test as developed by Conn and Fajans has been modified for field use.

The modified test consists of a single oral cortisone dose dependent on body surface (50 mg. per square

meter) given one and one-half hours before glucose loading. Thirty grams of glucose per square meter of body surface are given. Capillary blood samples are taken before glucose ingestion and at hourly intervals thereafter up to the third hour. The CGTT in its modified form was well tolerated by all patients, apart from rare instances of mild nausea.

The frequency distribution of the modified test in 116 "normal" volunteers has been determined and the statistical parameters of these distributions computed. The distributions are all unimodal, have their mode at the one-hour test period and are positively skewed at the one-, two- and three-hour test periods. No evidence of bimodality was found, so that, with the numbers tested, there is at present no objective dividing line between normal and abnormal.

A comparison of twenty diabetics (including three discovered during the study) and 116 normals at the different test periods of the CGTT showed that, on the basis of lack of overlap, the fasting stage used as a screening procedure would have allowed the exclusion of only 13 per cent from a suspicion of diabetes, while at the one-hour test period the proportion which could be excluded had increased to 80 per cent. Thus the contention of the U. S. Public Health Service is confirmed that the one-hour postglucose (or postprandial) test period is the most efficient period for diabetes screening purposes.

The CGTT was repeated in a group of thirty-one made up of every other male volunteer in the study. Repeatability was significant during the one-, two- and three-hour test periods.

Fifty-one male volunteers were given, in addition to the CGTT, a GTT identical in all respects but for the omission of cortisone. Correlation between GTT and CGTT was significant at all test periods.

Intra-test correlation between fasting, one-, two- and three-hour test periods was significant for the CGTT and the GTT series. The slopes of fitted regression lines of corresponding test periods did not vary significantly between the CGTT and the GTT. This is interpreted as meaning that the two tests do not differ qualitatively, e.g., measure the same physiologic or pathologic characteristics but on a different scale.

Maximal cortisone action was observed two and one-half hours after ingestion (one hour after glucose), when the absolute difference between average glucose levels during the CGTT and the GTT in the same persons reached 31.6 mg. per 100 ml. The difference decreased to 17.1 mg. per 100 ml. one hour later and to 11.2 mg. per 100 ml. two hours later. The relative effect of cortisone to raise average blood glucose levels at the same intervals after ingestion as referred to above were 20.9, 15.4 and 14.3 per 100 ml. respectively.

Comparison of simultaneously drawn capillary and venous blood specimens during the CGTT in thirteen persons showed a significant excess of capillary values one hour (average 24.0 mg. per 100 ml.) and two hours (average 13.3 mg. per 100 ml.) after glucose. The fasting and the three-hour test periods showed no difference.

It has been found that the mean glucose values of females exceed those in males at all test periods by 3 to 11 mg. per 100 ml., and that, with the numbers tested, the fasting and three-hour differences were statistically highly significant. This could either be due to a greater prevalence of carbohydrate metabolic disorders in females or else constitutes a physiologic characteristic which would require the setting of different diagnostic standards for the two sexes.

An unexplained, but repeated finding seen both in the CGTT and the GTT series, was a significantly higher peak value (one-hour test period) in whites when compared with nonwhites.

Older people gave higher one- and two-hour CGTT results than younger ones, but their fasting and three-hour levels were not different.

The results of urine glucose tests have been used for correlation purposes with the summed blood glucose levels. Accepting the upper 6 per cent of the summed glucose levels during the CGTT as the best estimate of true positive reactions, the urine test would have a sensitivity of 85.7 per cent and a specificity of 85.3 per cent.

SUMMARIO IN INTERLINGUA

Calibration de un Simplificate Test de Tolerantia pro Glucosa post Cortisona

Le test de tolerantia pro glucosa post cortisona, disveloppate per Conn e Fajans, ha essite modificate con le objectivo de render lo utilisabile in le campo.

In su forma modificate le test labora con un sol dose oral de cortisona de 50 mg per metro quadrate de superficie corporee administrate un hora e medie ante le cargation con glucosa. Trenta grammas de glucosa per metro quadrate de superficie corporee es administrate. Specimens de sanguine capillari es prendite ante le injection de glucosa e postea a intervallos de un hora usque al tertie hora. Le test de tolerantia pro glucosa post cortisona in su forma modificate esseva ben tolerate per omne le patientes.

Le sol qualification de iste assertion es que rar casos

de leve grados de nausea esseva notate.

Le distribution de frequentias del modificate test in 116 "normal" voluntarios esseva determinate, e le parametros statistic de iste distributiones esseva computate. Le distributiones es omnes unimodal, ha lor modos al periodo de un hora del test, e monstra dissymmetria positive al periodos de un, duo, e tres horas del test. Esseva trovate nulle evidentia de bimodalitate, de maniera que—a base del numero de casos testate—il non existe a iste tempore un linea objective de separation inter normal e anormal.

Un comparation de vinti diabeticos—incluse tres discoperite in le curso del presente studio—e 116 subjectos normal al varie periodos del test de tolerantia pro glucosa post cortisona monstra que—a base de non-superlappamento—le stadio jejun usate in le processo de triage haberea permittite le exclusion le exclusion de solmente 13 pro cento del casos ab le suspicion de diabete, durante que al periodo de un hora ille proportion habeva montate a 80 pro cento. Assi es confirmate le assertion del Statounitese Servicio de Sanitate Public que le periodo de un hora post glucosa (o post repasto) es le periodo le plus efficace pro le objectivos del triage pro diabete.

Le test de tolerantia pro glucosa post cortisona esseva repetite in un gruppo de trenta-un subjectos consistente de omne secunde voluntario mascule in le serie total. Le repetibilitate esseva significative al periodos de un, duo, e tres horas del test.

Cinquanta-un voluntarios mascule recipeva, a parte le test de tolerantia pro glucosa post cortisona, un test de tolerantia pro glucosa que esseva identic con le prime in omne repectos, excepte que le cortisona esseva omittite. Le correlation inte le duo tests esseva significative a omne periodos.

Le correlation intra-test inter le periodos jejun, de un, de duo, e de tres horas del test esseva significative pro le serie con cortisona e etiam pro le serie sin cortisona. Le pendentias de adaptate lineas de regression in correspondente periodos non variava significativemente inter le serie con cortisona e illo sin cortisona. Iste constatation es interpretate a significar que le duo tests non differe qualitativemente, i.e. que illos mesura le mesme characteristicas physiologic o pathologic, ben que a differente scalas.

Le activitate maximal de cortisona esseva observate duo horas e medie post le ingestion (un hora post le administration de glucosa), i.e. a un tempore quando le differentia absolute inter le nivellos medie de glucosa in le test con cortisona e illo sin cortisona in le mesme subjecto amontava a 31,6 mg per 100 ml. Iste differentia declinava a 17,1 mg per 100 ml un hora plus tarde e a 11,2 mg per 100 ml duo horas plus tarde. Le effecto relative de cortisona como augmentator del nivello medie de glucosa del sanguine al supra-mentionate periodos post le ingestion esseva 20,9, 15,4, e 14,3 mg per 100 ml, respectivemente.

Comparationes de simultaneemente obtenite specimens de sanguine capillari e venose, ab 13 subjectos durante le test de tolerantia pro glucosa post cortisona, monstrava un significative excesso del valores capillari post un hora (al media 24,0 mg per 100 ml) e post duo horas (al media 13,3 mg per 100 ml). In stato jejun e post tres horas a partir del ingestion de glucosa nulle differentia esseva constatate.

Esseva trovate que le valores medie de glucosa in femininas excede illos in masculos a omne periodos del test per inter 3 e 11 mg per 100 ml e que—con le numero de casos testate—iste differentia es statisticamente multo significative in stato jejun e post tres horas. Il es possibile que iste constatation se explica per un plus grande prevalentia de disordines del metabolismo de hydrato de carbon in femininas, sed il es etiam possibile que il se tracta hic de un characteristica physiologic que requirerea le establimento de differente standards diagnostic pro le duo sexos.

Un non-explicate sed repetitemente observate facto in ambe series (illo con e illo sin cortisona) esseva un significativemente plus alte valor maximal (periodo de un hora del test) in subjectos de racia blanc in comparation con non-blancos.

Personas de etates avantiate monstrava plus alte valores post un e duo horas, sed in stato jejun e post tres horas lor valores non differeva ab le valores pro subjectos de plus juvene etates.

Le resultatos de tests de glucosa urinari ha essite usate pro objectivos de correlation con le summate nivellos de glucosa sanguinee. Si nos accepta le 6 pro cento superior del summate nivellos de glucosa durante le test de tolerantia pro glucosa post cortisona como le melior estimation de ver reactiones positive, le test de urina ha un sensibilitate de 85,7 pro cento e un specificitate de 85,3 pro cento.

ACKNOWLEDGMENT

The study was aided by research contract No. SAph 73736 of the United States Public Health Service, Bureau of State Services, Chronic Disease Program, represented by Dr. Wilfred David and Mr. G. Remein.

The Department of Epidemiology of The Johns Hopkins University School of Hygiene and Public Health recruited a public health nurse, Mrs. N. M. Hayes, for the period of the study.

The Maryland State Department of Health made available the services of Mr. Thomas Sweeney, Jr., for field work connected with the study.

Dr. Victor McKusick, Professor of Medicine at The Johns Hopkins School of Medicine, paid out of funds at his disposal for the compensation of the prison volunteers.

The Laboratory of the Clinical Pharmacology Department Division of The Johns Hopkins University, Department of Medicine, (Director: Dr. L. C. Lasagna) was responsible for the glucose determinations, performed by the technicians, Mrs. Barbara Hooke and Mrs. Wanda Rudnicki.

The State of Maryland, Department of Correction, kindly gave pemission for the conduct of the study in volunteers of the male and female houses of correction in Jessup, Maryland.

Cortisone acetate was received free from The Upjohn Company, Kalamazoo, Michigan, and from Merck, Sharp and Dohme, West Point, Pennsylvania.

The authors wish to acknowledge gratefully the help and advice of Dr. Philip E. Sartwell, Professor of Epidemiology, The Johns Hopkins School of Hygiene; Mr. Jerome Cornfield, Professor of Biostatistics, The Johns Hopkins School of Hygiene; Dr. Claude J. Migeon, Associate Professor of Pediatrics, The Johns Hopkins School of Medicine; and Dr. Kenneth L. Zierler, Associate Professor of Medicine, The Johns Hopkins School of Medicine.

Particular gratitude and appreciation is due to all participating volunteers, faculty and students of The Johns Hopkins School of Hygiene and Public Health and inmates of the Houses of Correction for men and women in Jessup, Maryland.

REFERENCES

[1] Conn, J. W.: Interpretation of glucose tolerance test. Am. J. Med. Science *199*:555, 1940.

[2] Mosenthal, H. O., and Barry, Eileen: Criteria for and interpretation of normal glucose tolerance tests. Ann. Int. Med. *33*:1175, 1950.

[3] Moyer, John H., and Womack, C. Roy: Glucose tolerance: Comparison of four types of diagnostic tests in 103 control subjects and twenty-six patients with diabetes. Am. J. Med. Science *219*:161, 1950.

[4] Wilkerson, Hugh L. C., et al.: Diagnostic evaluation of oral glucose tolerance tests in nondiabetic subjects after various levels of carbohydrate intake. New England J. Med. *262*: 1047-53, 1960.

[5] Berger, H.: Method of increasing sensitivity of glucose tolerance test. J.A.M.A. *148*:364-66, 1952.

[6] West, Kelly M.: Comparison of the hyperglycemic effects of glucocorticoids in human beings: The effect of heredity on responses to glucocorticoids. Diabetes *6*:168-74, 1957.

[7] Fajans, S. S., and Conn, J. W.: An approach to the prediction of diabetes mellitus by modification of the glucose tolerance test with cortisone. Diabetes *3*:296-302, 1954.

[8] Fajans, S. S., and Conn, J. W.: Further studies on a test for the prediction of diabetes mellitus. Clin. Research Proc. *3*:122-23, 1955.

[9] Conn, J. W.: The Banting Memorial Lecture 1958. The prediabetic state in man: Definition, interpretation and implications. Diabetes 7:347-57, 1958.

[10] Fajans, S. S., and Conn, J. W.: The early recognition of diabetes mellitus. Ann. New York Acad. Sc. 82:208-18, Sept. 1959.

[11] Steinberg, A., and Wilder, R. A.: A study of the genetics of diabetes mellitus. Am. J. Genetics *4*:113, 1952.

[12] Wilkerson, H. L. C., and Krall, L. P.: Diabetes in a New England town. J.A.M.A. *135*:209, 1947.

Influence of Obesity on Morbidity and Mortality

Diabetes is perhaps the classic illustration of the association of overweight with disease, especially in the type of diabetes occurring in middle life and old age. The great majority of the patients are, or have been, seriously overweight, and a very high percentage truly obese. To take examples from recent studies, I may refer first to the data on ninety industrial workers. Of these, nearly 50 per cent were 20 per cent or more overweight at the time of diagnosis. If the weight before onset were used, the proportion would be appreciably greater. In a recent study in a German clinic, 53 per cent of the patients were appreciably overweight according to the standard used.

The high mortality from liver disorders in the studies of insured overweights is paralleled by their high incidence in clinical experience. A recent study of twenty hospitalized men who were 50 per cent over ideal weight, with no illness involving or likely to involve the liver, showed that six of them had enlarged livers, five had enlarged spleens and four of these had both conditions. Nine of them had impaired glucose tolerance. Moreover, liver function tests were frequently abnormal. In another study of eighteen obese patients in a Veterans Administration Hospital, a significant proportion were found to have abnormal liver function tests and a great many had elevated blood sugars on a glucose tolerance test.

By Herbert H. Marks in *Bull. New York Acad. Med.*, Second Series, Vol. 36, No. 5, p. 305, May 1960.

Granulation and Reactive Zinc in the Cells of the Islets of Langerhans

Effect of Prolonged Insulin Treatment

J. Logothetopoulos, M.D., J. Kraicer, M.D., and C. H. Best, M.D., D.Sc., F.R.S., Toronto

The cells of the islets of Langerhans in most species contain a high concentration of reactive zinc. This has been demonstrated by histochemical methods applied to sections of fixed and unfixed pancreas[1-3] and, more satisfactorily, by the chelation of intracellular zinc after the infusion of diphenylthiocarbazone (dithizone).[4,5]

The intracellular zinc of the beta cells has been reported to be decreased after hyperglycemia produced by glucose injections, by adrenaline and by a high carbohydrate diet.[2,5,7] Beta-cell granulation in stained histological sections is also markedly decreased after the application of these stimuli for beta-cell secretion.[8-12] A marked reduction in extractable insulin and beta-cell granulation has also been found in rats and other animals when adequate amounts of insulin have been injected over prolonged periods.[9,13,14] In contrast to the hyperactivity of the beta cells after glucose stimulation, morphological evidence of inactivity of the beta cells has been associated with insulin administration.[15] In the present investigation the content of zinc in the alpha and beta cells of the islets was studied in rats after prolonged administration of insulin, and this was compared with the degree of granulation in these cells in fresh-frozen and stained sections of the pancreas. Further insight into the relationship between zinc and granulation was sought by correlating histophotometric estimations of both for fifteen days following the cessation of insulin administration.

METHODS AND PROCEDURES

Animals

Young adult female Wistar rats weighing 190 to 220 gm. were used. Protamine Zinc Insulin was injected daily, late in the afternoon, in progressively increasing amounts. A daily dose of 5 to 7 units was reached by the end of four to five weeks. This dose was maintained for four to six weeks. In one series of rats, an additional injection of NPH insulin was given in the morning (2 to 3 units). Deaths due to hypoglycemia occurred at all dosage levels. Approximately half the rats were dead by the end of the second month. The animals were kept in groups of four or five with free access to ground chow, supplemented after each injection of insulin with brown bread.

Control rats were given saline injections and had access to the same food. A group of fifteen treated rats and a similar number of untreated rats were placed in metabolic cages to permit an estimation of food intake and glucosuria after cessation of the injections.

Treated and control rats were sacrificed daily for a period of twenty days following the cessation of insulin injections. A number of them were infused with dithizone (for the demonstration of islet-cell zinc) immediately before killing.

Blood sugar

Blood sugars were estimated on samples obtained from the cut tail after gently warming the animals in a heated box.

Intravital chelation of reactive zinc

The dithizone solution was prepared as follows: A weighed amount of dithizone was dissolved in a small volume of absolute alcohol with drops of N. NaOH. This solution was then added slowly to boiling distilled water. Drops of N/10 hydrochloric acid were then added, with continuous stirring, until a permanent precipitate (one third to one half of the initial amount) of dithizone was formed. Gentle boiling was continued until three quarters of the original volume of water remained. The final concentration of dithizone in the weakly alkaline solution was estimated by collecting the precipitate on a filter paper, weighing it after drying, and subtracting its weight from the amount initially dissolved.

The concentrations of the solutions used for injection varied from 5 to 10 mg. per milliliter. Under light ether anesthesia a fine polythene tube was introduced into one of the jugular veins and secured on the ipsilateral

From the Banting and Best Department of Medical Research, Charles H. Best Institute, University of Toronto, Toronto, Ontario, Canada.

ear and back of the animal. Two hundred and twenty-five to 250 mg./kg. body weight of dithizone was infused continuously with a syringe pump in thirty to forty minutes. This dose, given at the above rate, produced a deep-green coloration of subcutaneous and abdominal fat and a maximal reaction in the cells of the islets.

Histological technics

At the end of the infusion of dithizone, the rat was decapitated and samples were taken from the splenic portion of the pancreas. Fresh-frozen sections at 25 μ and 7.5 μ were obtained by a cold-knife technic. This procedure was carried out in a cold room at 0° C. Sections, still frozen, were gently unfolded on the precooled knife with fine brushes and rapidly mounted by contact on carefully cleaned slides. The mounted, uncovered sections were kept in the dark in air-tight slide boxes at 4° C. The intensity of the zinc reaction did not fade for two to three hours. The section on the slide was mounted in neutral glycerol under a thin coverslip immediately before microscopic observation or histophotometric evaluation.

Pancreatic tissues of insulin-treated and control rats which did not receive dithizone were similarly processed. A portion of the pancreas fixed in Bouin's solution was embedded in paraffin and cut at 5μ. Several batches of aldehyde fuchsin solutions were prepared and tested daily on slides of normal rat pancreas. Only solutions which stained the beta cells intensely and the acinar tissue very faintly were used to stain pancreatic sections from the control and insulin-treated animals. Oxidation time with acid permanganate, washing and dehydration times in alcohol, were kept constant.

Histophotometric measurements

General. The eyepiece of the straight tube of a Leitz-dialux microscope was replaced by a special ocular eyepiece shown schematically in figure 1. A diaphragm (d) with a central round aperture 2 mm. in diameter and a reflecting undersurface was incorporated at the level of the formation of the primary image. Thus rays corresponding to the center of the primary image (center of field of the object) pass through the aperture and strike the overlying current-generating photocell (p.c.). Rays forming the periphery of the primary image are reflected and produce a magnified image of the field of view with a central black circle in the side eyepiece (e.p.).

The photocell current was fed through a variable resistor to a string galvanometer. The deviations of the mirror were read on a meter scale at a distance of three meters. A regulated 12 v. battery supply was

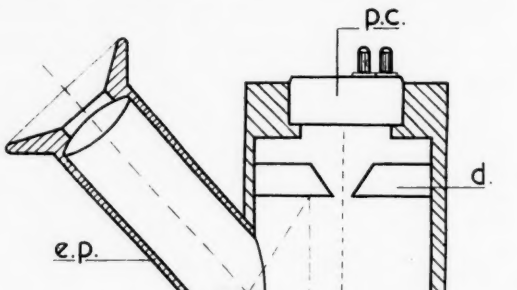


FIG. 1. Ocular eyepiece with photocell (modified Dr. B. Lange, Berlin).

used for illuminating the 6 v. 5 amp. bulb of the microscope.

A green filter with a narrow band of maximal tansmittance at 530 mμ was used (maximal absorption of zinc-dithizonate 525 mμ) for the estimation of the zinc-chelation reaction. The same filter was found satisfactory for the islets stained with aldehyde fuchsin. Kohler illumination conditions were used.

Histophotometric estimation of zinc. After adding glycerol each frozen section which had been kept in the dark was covered by a coverslip and placed on the stage of the microscope. Consecutive fields were observed through a $\times$10 objective and the inclined binocular arm of the microscope. The islets stood out distinctly against a transparent background because of the black dithizonate granules in their cells. The islet boundaries were sharply delineated by the intensely stained rim of peripheral alpha cells. Photometric readings were obtained from islets, the diameter of which was at least twice that of the aperture of the diaphragm as seen in the field of view of the special ocular. When such an islet was brought into view, it was centered with a 40$\times$ objective in the field of view of the special eyepiece. The stage was then moved horizontally so that a transparent neighboring area of acinar tissue filled the aperture. The light spot of the galvanometer was set at 100. The islet was then brought back into the field and a reading from the center of the islet taken. Thus the light transmittance of the beta-cell area was expressed as a percentage of the transmittance of the

transparent acinar tissue.

Five to ten islets were measured on each slide and a total of fifty islets measured for each rat. There was a remarkably narrow range of readings from different slides of the same rat. This demonstrates the small variation in section thickness and the stability of the zinc dithizone complex when sections are kept in the dark at 4° C.

The sources of error and the statistical validity of this method are thoroughly discussed by Wolff et al.[5] It should be stressed that in our experiments control rats were always injected under the same conditions as the experimental ones and their pancreases processed in exactly the same way. The histophotometric values are relative and do not represent absolute measurements of zinc dithizonate.

Histophotometric estimation of aldehyde-fuchsin staining granules. The same methodology was applied to the paraffin sections stained with aldehyde fuchsin. The transmittance through the islet was expressed as a percentage of the transmittance through a tissue-free area of the slide. This was found desirable for these comparative studies since the acinar tissue stained slightly and variably with aldehyde fuchsin.

RESULTS

Body weight, food intake

Rats receiving insulin ate 20 to 30 per cent more food and, within a period of two months, their body weights were 30 to 80 per cent higher than those of the control group. Immediately after cessation of insulin injections, the food intake of the treated rats fell to 5 to 20 per cent of the level of the untreated controls and most of their additional weight was lost during the first three weeks.

Blood glucose

Blood glucose levels during the period of injections were measured every ten days, in eight to ten randomly selected rats, 4, 8, 16, 20 and 24 hours after the afternoon injection. Even when the high doses (5 to 7 units) had been reached a large scatter of blood sugar values was found at 16, 20 and 24 hours following the insulin injection (figure 2). In a later series of rats an additional morning injection of NPH insulin was given to lengthen the hypoglycemic phase during the twenty-four hours.

A variety of patterns of blood glucose levels was found after the cessation of treatment (figure 3). Hyperglycemic values were found for two to four days in spite of the marked decrease in food intake. When treated rats were force-fed during this period they became severely glycosuric with very high blood sugar

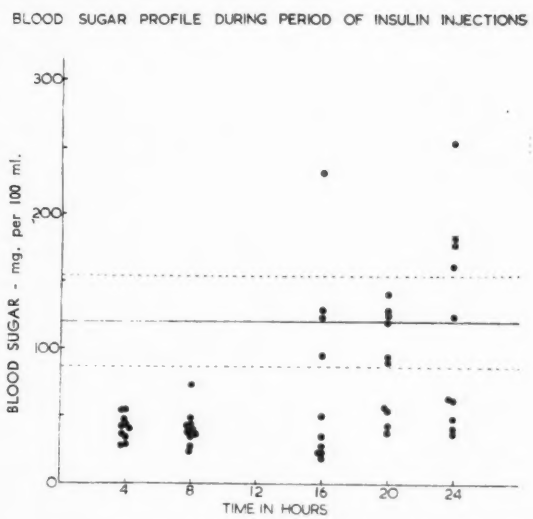


FIG. 2. Blood sugars of randomly selected rats following the injection of 5 units of Protamine Zinc Insulin (thirty-fifth day of insulin treatment). Solid and broken horizontal lines: Mean ± two S.D. of blood sugars from forty control rats.

values. Controls force-fed the same amount of food did not exhibit glucosuria or severe hyperglycemia. During this phase of transient severe diabetes many of the force-fed rats died.

Histologic observations

Fixed preparations: Aldehyde fuchsin. The beta cells of the islets of the insulin-treated rats were of small size, and appeared crowded. Their nuclei were small. The degree of beta-cell degranulation varied among individuals (figures 6, 7). In some of the rats, islets free of granules could scarcely be distinguished in the midst of the acinar tissue. In other rats scarce aldehyde fuchsin staining granules were present at the vascular end and at the periphery of most beta cells. The islet tissue in many of the rats showed less light transmittance than the acinar tissue. For this reason and because of the variable background staining of the acinar tissue, the light transmittance of islets was expressed as a percentage of light transmittance through tissue-free areas of the slides.

There was no increase in beta-cell granulation during the first three days after cessation of insulin injection. This was the period of transient diabetes. Within the subsequent three days there was a steep rise in granulation. From the seventh day onward the values for transmittance fell within the range of control animals (figure 4).

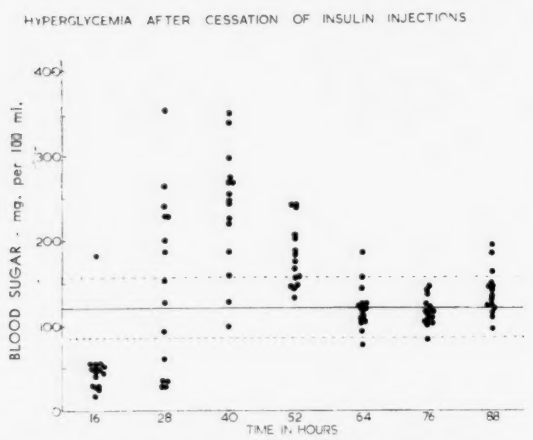


FIG. 3. Transient diabetes following insulin treatment for two months. Food intake was markedly decreased during this period. Solid and broken horizontal lines as in figure 2.

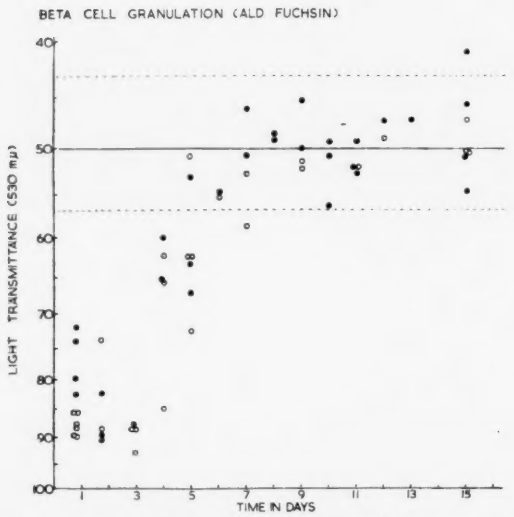


FIG. 4. Ordinate: Relative light transmittance (semilog. scale) after standardized aldehyde fuchsin staining. Means of fifty islets from one rat are plotted.
Abscissa: Days after cessation of treatment.
Solid circles: Dithizone infused prior to sacrifice. Open circles: no dithizone infusion. Solid and two horizontal broken lines: Mean ± two S.D. of values obtained from twenty control rats.

The intensity of aldehyde fuchsin positive granulation did not appear to be different in rats which received an infusion of dithizone prior to sacrifice (figure 4).

Fresh-frozen sections. Phase contrast and dark-field illumination

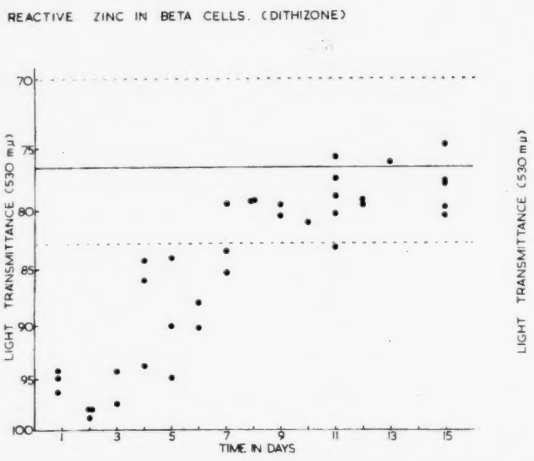


FIG. 5. Ordinate: Light transmittance (semilog. scale) of islets after intravital zinc chelation with dithizone.
Abscissa: Days after cessation of insulin treatment.
Each solid circle represents the mean value of fifty islets from one rat. Solid and broken horizontal lines: Mean ± two S.D. of values from fifteen control rats.

The appearance of the alpha and beta cells of the islets of normal rats in fresh-frozen sections mounted in neutral glycerol and observed under phase contrast and dark-field illumination is the reverse of that described in the rabbit.[20] In the rat the granules of the beta cells stand out sharply (figure 8). The cytoplasm of the alpha cells appears empty. In the fresh preparations, no granules were found during the first three days after the cessation of treatment (figure 9). The patterns of granulation observed after this initial period paralleled the density of granulation as observed in the aldehyde fuchsin stained material.

Intravital zinc chelation reaction

After the intravenous infusion of dithizone the islets of Langerhans appeared as pink spots in the midst of acinar tissue. The color was paler in the insulin-treated rats.

In fresh-frozen sections mounted in glycerol the zinc dithizonate precipitate appeared as violet granules when examined with transmitted light and as highly refractile orange granules when sections were examined under dark field illumination (figure 10). Alpha cells were densely filled with the colored precipitate in control animals. The reaction was weaker in the beta cells as reported by Wolff et al.[4]

In insulin-treated rats alpha cells were also filled with the precipitate but there was no reactive zinc in the beta cells (figure 11). Areas of beta cells were nearly as transparent as acinar tissue.

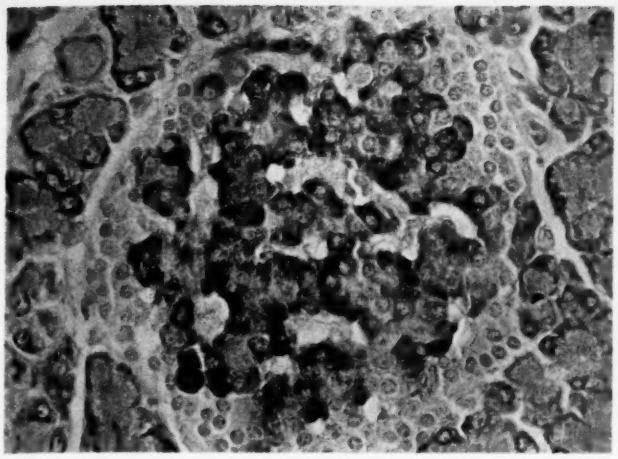


FIG. 6
Pancreatic islet of control rat. Aldehyde-fuchsin, Haemalum. Fully granulated beta cells. X300

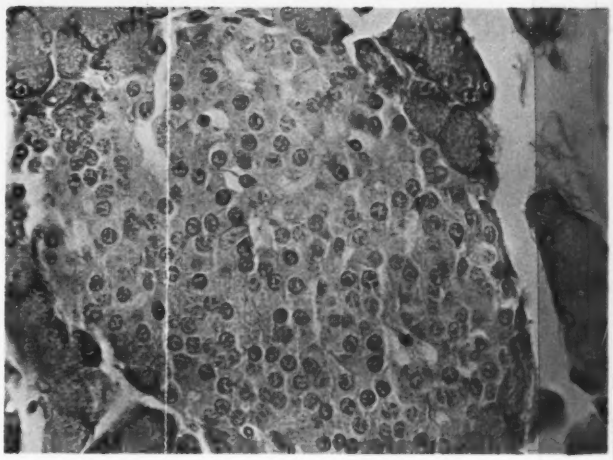


FIG. 7
Pancreatic islet of insulin treated rat. Aldehyde-fuchsin, Haemalum. Degranulation of beta cells. X320

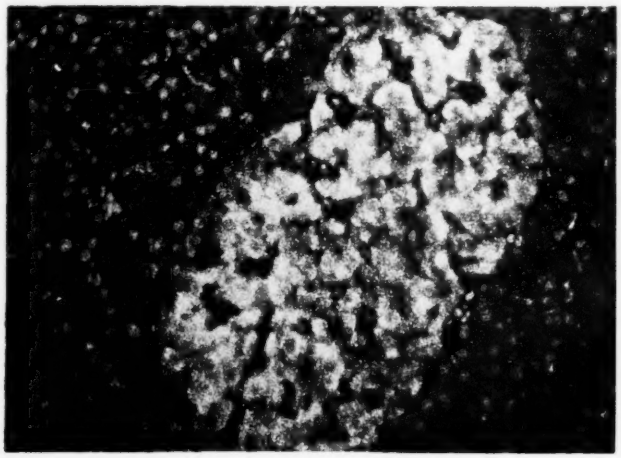


FIG. 8
Pancreatic islet of control rat. Fresh-frozen section mounted in glycerol. Dark-field illumination highly refractile granules in the beta cells. Only nuclei of alpha cells and acinar cells show luminescence. X200

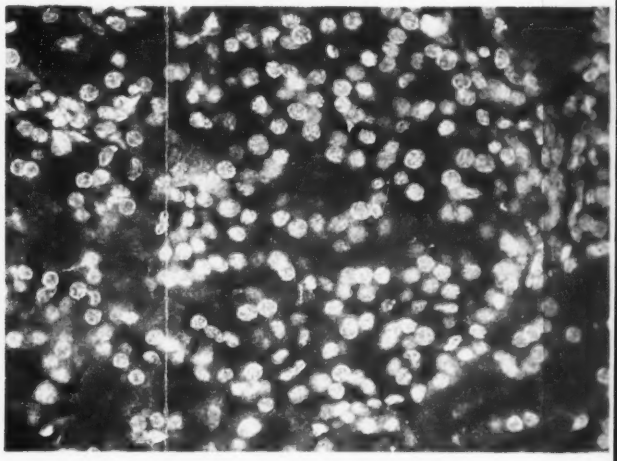


FIG. 9
Pancreatic islet of insulin treated rat. Fresh-frozen section mounted in glycerol. Dark-field illumination. Complete absence of beta cell granules. X300

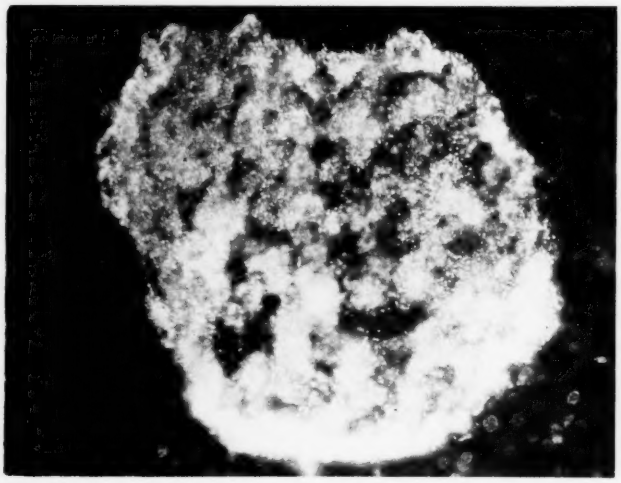


FIG. 10
Pancreatic islet of control rat infused with dithizone. Fresh-frozen sections mounted in glycerol. Dark-field illumination. Granular precipitate of zinc-dithizonate in peripheral alpha cells (+++) and beta cells (+). Photomicrography does not reproduce orange color of zinc-dithizonate granules. X230

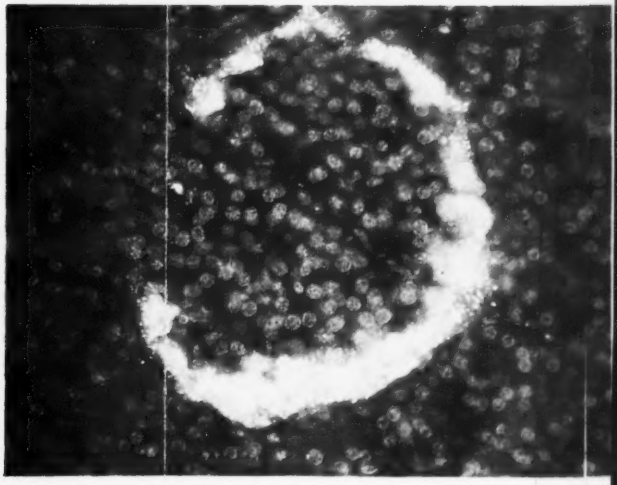


FIG. 11
Pancreatic islet of insulin treated rat infused with dithizone. Fresh-frozen sections mounted in glycerol. Dark-field illumination. Granular precipitate of zinc-dithizonate in the peripheral alpha cells only. Beta cells contain no reactive zinc or secretion granules. X200

The reaccumulation of reactive zinc in the beta cells, after cessation of the insulin treatment, followed closely the pattern of return of beta-cell granulation, a steep rise being found between the third and seventh day (figure 5).

DISCUSSION

Morphological criteria indicated that the activity of the beta cells was strongly depressed after the prolonged administration of insulin. The morphological changes were associated with an inhibition of insulin secretion since a phase of transient diabetes followed the cessation of the injections.

Normal or elevated blood sugar values were found for intervals of various duration prior to each daily Protamine Zinc Insulin injection, suggesting that the inhibition of the beta cells may not have been maximal.

There is abundant evidence to support a close correlation between extractable insulin and the extent of granulation of the beta cells.[13, 18] It, however, does not necessarily follow that insulin is the only structural material of the beta-cell granule.[19] Other proteins may also contribute to it.

During the transient diabetic phase there was no increase in the granulation of the beta cells. The rate of the synthesis and liberation of insulin, at the cessation of the injections, failed, for three to four days, to maintain normal blood sugar values.

The biosynthetic processes recovered rapidly after the third day and were able to provide sufficient insulin and other "material" for storage in the form of granules, and insulin for metabolic needs. The sharp rise in granularity of the beta cells coincides with the establishment of normoglycemic levels. By the seventh day the extent of granularity was within the range of the control rats. A close correlation was found between granularity demonstrated in fresh unfixed preparations and that seen in sections stained with aldehyde fuchsin. Degranulation of beta cells therefore is not due to a loss of the stainability of existing granules.

Fresh-frozen sections of the rat pancreas examined with phase contrast and dark field revealed distinct beta-cell granules but no alpha-cell granules. This, as noted before, is the reverse of findings in rabbit islets.[20] This fact adds to the existing body of evidence that there are structural differences between the granules in the two types of islet cells as well as between islet cell granules in different species.

The process of regranulation followed closely the pattern of the reappearance of zinc. This points to the significance of zinc in the beta cells as a structural component of the storage granule and not as an index of activity of the cell. Both stimulation by glucose and prolonged inhibition by exogenous insulin lead to depletion of zinc.

The work of Maske demonstrated clearly the relationship of zinc to beta-cell granules.[21] Insulin and zinc were highly concentrated in the particulate fraction of homogenates of the principal islets of fishes.

However, the colored granules seen in the alpha and beta cells after the injection of dithizone do not represent, according to our observations, pre-existing granules in the beta cells. Granules of the same size and with the same optical properties can be seen at any site (subcutaneous tissue) in which a solution of zinc has been injected prior to an infusion of dithizone.[22] They can also be produced in vitro by slowly mixing weak solutions of dithizone and zinc chloride.[22] They represent therefore only the product of precipitation of reactive zinc with dithizone.

The intensity of the staining reaction with aldehyde fuchsin was not affected by the precipitation of zinc resulting from a preceding infusion of dithizone. Zinc does not seem to contribute to this staining reaction and an infusion of dithizone is not followed by a quick disintegration of the granules.

The alpha cells had a normal appearance in these insulin-treated rats. There was no obvious change in their number or their zinc content. This makes it difficult to attribute to them a significant role in the compensatory reactions to insulin hypoglycemia.

SUMMARY

Protamine Zinc Insulin was injected daily, in progressively increasing amounts, into adult female rats over a period of two months. Treated and control rats were killed daily for fifteen consecutive days after the cessation of the insulin injections. Reactive zinc in the alpha and beta cells was demonstrated by intravital chelation with dithizone. Beta-cell granulation was evaluated in fresh-frozen sections examined under dark-field and phase-contrast illumination and in paraffin sections stained with aldehyde fuchsin. Relative histophotometric measurements were made of the intensity of the zinc dithizonate intracellular precipitate and of the granules stained with aldehyde fuchsin.

The following observations were made:

1. A loss of reactive zinc was found in the extensively degranulated beta cells after insulin treatment.

2. There was no increase in the granulation or in the zinc content of the beta cells for three days following the cessation of the insulin injection. This was a period of transient diabetes.

3. A steep rise in beta-cell granulation and in intra-

cellular zinc occurred between the third and seventh day. Normal histophotometric levels were found after this period.

4. Dithizone infusion did not affect the stainability of beta-cell granules stained with aldehyde fuchsin.

5. There was no morphological change or loss of zinc content of the alpha cells after insulin treatment.

SUMMARIO IN INTERLINGUA

Granulation e Zinc Reactive in le Cellulas del Insulas de Langerhans: Effecto de un Prolongate Therapia a Insulina

Insulina a zinc e protamina esseva injicite diurnemente, in doses progressive, a rattas adulte durante un periodo de duo menses. Post le cessation del injectiones de insulina, rattas in le gruppo experimental e in un gruppo de controlo esseva sacrificate in dece-cinque consecutive dies. Zinc reactive in le cellulas alpha e beta esseva demonstrate per chelation intravital con dithizona. Granulation del cellulas beta esseva evalutate in sectiones ab specimens congelate in stato fresc per medio de examines a campo obscur e illumination a contrasto de phase e in sectiones in paraffin tincturate con aldehyda-fuchsina. Relative mesurationes histophotometric esseva facite del intensitate del precipitato intracellular de dithizonato de zinc e del granulos tincturate a aldehyda-fuchsina.

Esseva facite le sequente observationes:

1. Un perdita de zinc reactive esseva constatate in le extensemente disgranulate cellulas beta post le tractamento con insulina.

2. Esseva trovate nulle augmento del granulation o del contento de zinc in le cellulas beta durante tres dies post le cessation del injection de insulina. Isto esseva un periodo de diabete transiente.

3. Un augmento acute in le granulation del cellulas beta e in lor contento de zinc occurreva inter le tertie e le septime die. Post iste periodo, normal nivellos histophotometric esseva incontrate.

4. Le infusion de dithiozona non afficeva le tincturabilitate del granulos del cellulas beta in le tincturation con aldehyda-fuchsina.

5. Esseva constatate nulle alteration morphologic e nulle perdita in le contento de zinc del cellulas alpha post le tractamento con insulina.

ACKNOWLEDGMENT

This work was supported in part by Consolidated Grant No. 2 from the National Research Council of Canada. The authors wish to thank Mr. T. Gardner and Mrs. A. Gardner for competent technical assistance.

REFERENCES

[1] Okamoto, K.: Biologische Untersuchungen der Metalle. (VI Mitteilung.) Histochemischer Nachweis einiger Metalle in den Geweben, besonders in den Nieren und deren Veränderungen. Tr. Soc. Path. Jap. *32*:99-105, 1942. (In Okamoto, K.: Experimental pathology of diabetes mellitus. Tohoku J. Exp. Med. *61*:Suppl. 3, 1955.)

[2] Okamoto, K.: Biologische Untersuchungen der Metalle. (VII Mitteilung.) Ueber das Gewebseisen der Malarialeber und -milz, die Zinkverteilung im Tierreich und den Zinkstoffwechsel. Tr. Soc. Path. Jap. *33*:247-52, 1943. (In Okamoto, K.: as above.)

[3] McNary, W. F., Jr.: Zinc-dithizone reaction of pancreatic islets. J. Histochem. Cytochem. *2*:185-95, 1954.

[4] Wolff, H., Maske, H., Stampfl, B., and Baumgartea, F.: Untersuchungen über den Dithizondiabetes. Naunyn Schmiedeberg Arch. Exp. Path. *216*:440-56, 1952.

[5] Wolff, H. P., Ringleb, D., and Amann, R.: Histochemische Untersuchungen über das Inselzink. Ztschr. f.d. ges. exper. Med. *126*:390-416, 1955.

[6] Best, C. H., and Haist, R. E.: The effect of insulin administration on the insulin content of the pancreas. J. Physiol. *100*: 142-46, 1941.

[7] Maske, H., Wolff, H., and Stampfl, B.: Ueber die Verhinderung der diabetogenen Alloxanwirkung durch vorhergehende Glucosegaben. Klin. Wchschr. *31*:79-81, 1953.

[8] Wissler, R. W., Findley, J. W., Jr., and Frazier, L. E.: Pancreatic islet hyperplasia in rats force fed high carbohydrate diets. Proc. Soc. Exper. Biol. & Med. *71*:308-13, 1949.

[9] Haist, R. E.: Factors affecting the insulin content of the pancreas. Physiol. Rev. *24*:409-44, 1944.

[10] Gomori, G., Friedman, N. B., and Caldwell, D. W.: Beta cell changes in guinea pig pancreas in relation to blood sugar level. Proc. Soc. Exp. Biol. & Med. *41*:567-70, 1939.

[11] Woerner, A. C.: Studies of the islands of Langerhans after continuous intravenous injection of dextrose. Anat. Rec. *71*:33-58, 1938.

[12] Barron, S. S., and State, D.: Effect of prolonged intravenous administration of dextrose on beta cells of islets of Langerhans. A.M.A. Arch. Path. *48*:297-304, 1949.

[13] Bell, E. T.: The incidence and significance of degranulation of the beta cells in the islets of Langerhans in diabetes mellitus. Diabetes *2*:125-29, 1953.

[14] Latta, J. S., and Harvey, H. T.: Changes in the islets of Langerhans of the albino rat induced by insulin administration. Anat. Rec. *82*:281-96, 1942.

[15] Batts, Adrienne: Use of the Golgi apparatus as an indicator of the level of activity of the cells of the islets of Langerhans. Ann. New York Acad. Sc. *82*:302-18, 1959.

[16] Gomori, G.: Aldehyde-fuchsin: A new stain for elastic tissue. Am. J. Clin. Path. *20*:665-66, 1950.

[17] Wilson, W. D.: Differential cytological staining of anterior pituitary and islets of Langerhans. Anat. Rec. *112*:468, 1952.

[18] Hartroft, W. S., and Wrenshall, G. A.: Correlation of beta-cell granulation with extractable insulin of the pancreas. Diabetes *4*:1-7, 1955.

[19] Bangle, R., Jr.: Factors influencing the staining of beta-cell granules in pancreatic islets with various basic dyes, including paraldehyde-fuchsin. Am. J. Path. *32*:349-62, 1956.

[20] Logothetopoulos, J., and Salter, J. M.: Morphology and cytochemistry of alpha cells of the rabbit pancreas. Diabetes *9*:31-37, 1960.

[21] Maske, H.: Interaction between insulin and zinc in the islets of Langerhans. Diabetes *6*:335-41, 1957.

[22] Logothetopoulos, J.: Intravital chelation of zinc in the prostate of the rat. Am. J. Path. *37*:357-75, 1960.

Serum Glycoproteins in Newborn Infants of Diabetic Mothers

Otakar V. Sirek, M.D., Ph.D., Anna Sirek, M.D., Ph.D., and Bernard S. Leibel, M.D., Toronto

The behavior of the protein-bound carbohydrates in blood of diabetic patients has been the subject of a number of investigations.[1-9] The consensus of opinion is that the level of certain glycoprotein fractions becomes appreciably elevated at the time that clinical manifestations of degenerative vascular lesions are present. This is in keeping with the experience gained from a variety of disease states in which elevation of serum glycoproteins is associated with distinct alterations in the structure of connective tissue.[10] Otherwise, little is known with certainty about the effect of the diabetic state upon the protein-bound carbohydrates in blood.

The origin of many glycoproteins and the mechanism of maintaining their normal levels are both unknown, but it has been proposed that a rise in serum glycoproteins may be an expression of tissue proliferation.[11-13] It also has been suggested that an increase in concentration may be a consequence of tissue destruction.[14] The concept of excessive depolymerization of the amorphous ground substance as the cause of an elevation of circulating glycoproteins[15] and also the suggestion that inflammation may stimulate overproduction[16] have experimental evidence in their favor. Nevertheless, a rise in circulating glycoproteins is sometimes encountered under circumstances which make it appear as though larger amounts were in transit to tissues. The glycoproteins rise in response to tissue repair after injury[17] and during regeneration of plasma proteins after hemorrhage;[18] they fall during dietary protein depletion and rise during repletion.[19] In a number of animal species but not in man, fetal blood contains large amounts of the glycoprotein fetuin.[20-22] Glycoproteins rise in women during the later stages of pregnancy[12,13] and seem to rise in response to new formation of tissue in general.[11,23] Infants of diabetic mothers have a tendency to macrosomia as well as to splanchnomegaly[24,25] and therefore it seemed of interest to investigate their glycoprotein levels at birth.

From the Department of Physiology and the Banting and Best Department of Medical Research, University of Toronto, Toronto, Canada.

Determinations of total serum protein-bound hexose, hexosamine and sialic acid in blood of twenty infants of nondiabetic mothers and seventeen infants of diabetic mothers revealed that the amounts of these constituents were indeed significantly increased in the latter group of infants. The diabetic women showed no abnormal elevation of serum glycoproteins when compared with nondiabetic pregnant women at the time of delivery.

MATERIAL AND METHODS

Each diabetic woman who was selected for this study had a history of an uneventful pregnancy without episodes of ketoacidosis or hypoglycemia and showed no clinical manifestations of vascular complications. The duration of diabetes varied from a few months to twenty years with an average of ten years. Nine patients were delivered by elective cesarean section, and in eight patients labor was either spontaneous or was induced by rupturing membranes before term. The controls consisted of nondiabetic normal women who were delivered before term by elective cesarean section for pelvic disproportion. Most of the women were multigravidas, and only six in the diabetic series and four in the normal series were primigravidas. The average length of gestation (calculated from the first day of the last menstrual period) was 37.3 weeks for the diabetic women and 38.5 weeks for the nondiabetic women. All infants survived, had no evidence of congenital malformations and were discharged from hospital with a good prognosis.

Maternal blood was obtained by venipuncture of the antecubital vein one-half to one hour before delivery. Blood samples from infants at birth were obtained by needle from the placental end of the umbilical cord. Clotting and hemolysis due to manipulation were prevented by using heparin in minute measured amounts which did not change the results of chemical determinations of hexose and hexosamine by more than + 0.5 mg. per cent. This was confirmed by analyses of sera and plasma prepared from a number of blood specimens. All plasma samples were frozen and processed within the

next three to four months. The thawed specimen contained a fibrin clot which was removed before aliquots were taken for chemical analyses and results are therefore expressed as serum concentrations.

Total protein-bound hexose was determined with the orcinol reagent according to Lustig and Langer[26] modified by Weimer and Moshin.[27] An aqueous mannose-galactose solution 1 : 1 was employed as standard. Hexosamine was estimated by the Elson-Morgan procedure in Rimington's modification as described by Winzler.[10] Sialic acid was determined by the tryptophan-perchloric acid procedure of Seibert et al.[28] and also by the thiobarbituric acid assay of Warren.[29] The methods were standardized against crystalline N-acetyl-neuraminic acid and an alpha-one glycoprotein preparation of known sialic acid content was employed as routine standard.* Because the procedure of Warren measures only free sialic acid, 0.01 or 0.02 ml. of serum were mixed with 0.2 ml. or 1 N HCl and placed in a boiling water bath for exactly three minutes; prolonged boiling destroys the liberated sialic acid. Seromucoid was determined according to the procedure recommended by Weimer and Moshin[27] which is a modification of the original method of Winzler et al.[30] The results are expressed as hexose, which was determined with the orcinol reagent.[26,27] All determinations were performed in duplicate and repeated analyses were carried out on a number of specimens which were kept frozen in aliquots up to six months. Results agreed with the average values within ± 4 per cent. Major difficulties were encountered with the tryptophan-perchloric acid procedure for the determination of sialic acid. It lacked specificity and reproducibility; the latter could be somewhat improved by including a serum sample as a secondary standard. The final results were adjusted to the over-all average obtained with the standard serum sample.

Total serum proteins were determined by the method of Weichselbaum.[31] Paper electrophoresis of serum proteins was carried out in a veronal buffer of *p*H 8.6 according to Kunkel.[32] The strips were stained with bromophenol blue and cut into appropriate segments. The dye was eluted with 0.01 N NaOH and the intensity of the color was measured in a photoelectric colorimeter. Strips were also stained for protein-bound carbohydrates according to Björnsjö.[33] The stained patterns were not quantitatively analyzed and only photographs were kept as permanent records. Serum cholesterol was determined according to Bloor, Pelkan and Allen.[34] The test for C-reactive protein was carried out according to Anderson and McCarthy[35] using commercially available anti-serum (Schieffelin & Co.). Glucose was estimated in heparinized whole blood according to King[36] and microhematocrit determinations were carried out in capillary tubes (A. H. Thomas & Co.). The data were analyzed statistically by Fisher's *t* test.[37]

*The gifts of crystalline N-acetyl-neuraminic acid from Prof. G. Blix, Sweden, and of the glycoprotein standard from Dr. S. H. Jackson, Toronto, are gratefully acknowledged.

RESULTS

The results of determinations of a number of blood constituents other than protein-bound carbohydrates in diabetic and nondiabetic pregnant women prior to delivery are presented in table 1. There was no statistically significant difference between the two groups of patients with respect to hematocrit, cholesterol and total protein. In the diabetic group the mean blood sugar value was not abnormally high but due to sampling under conditions which were not constant the values varied widely from patient to patient. Results obtained from electrophoretic separation of serum proteins indicated that the albumin level was slightly decreased and the alpha-two globulin fraction increased in the diabetic group of patients.

The amounts of circulating glycoproteins at the time of delivery expressed as hexose, hexosamine and sialic acid are presented in table 2. The values for all three carbohydrate constituents determined in the total glycoprotein moiety were similar in both groups in spite of the above-mentioned elevation of the alpha-two globulin fraction which is known to be rich in carbohydrate.[14] It is interesting that in the absence of any difference in alpha-one globulin levels the seromucoid hexose[10] gave significantly lower values for the diabetic women than for the nondiabetic women.

C-reactive protein was present in seven out of twelve diabetic women tested and was also positive in ten out of fourteen normal pregnant women. A positive C-reactive protein test in pregnancy was also observed by other authors.[13,38] It has been reported that a rise in sialic acid levels under a variety of conditions is accompanied frequently with the presence of C-reactive protein in serum.[39] It is perhaps of interest to mention here that the sialic acid levels of our pregnant diabetic and nondiabetic women were grossly elevated when compared with values obtained in six nonpregnant women of childbearing age. The mean values for sialic acid were 71.6 $\pm$ 3.2 mg. per cent by the tryptophan-perchloric acid procedure and 58.5 $\pm$ 3.1 mg. per cent by the thiobarbituric acid method. A similar but less dramatic increase in diabetic and nondiabetic pregnant women was found for total protein-bound hexose, while no appreciable changes were found in glucosamine concentrations.

TABLE 1

Blood chemistry of patients antepartum

Constituent	Mean ± S.E. (No. of observations) Diabetic	Normal	t	P
Hematocrit (per cent formed elements)	39.6 ± 1.3 (9)	39.1 ± 1.3 (11)	0.279	>0.5
Blood sugar (mg./100 ml.)	207 ±66 (9)	109 ±19 (11)	1.557	<0.2
Cholesterol (mg./100 ml.)	299 ±23 (12)	338 ±23 (14)	1.189	>0.2
Total protein (gm./100 ml.)	6.05 ± 0.12 (12)	5.81 ± 0.13 (14)	1.380	<0.2
Albumin (gm./ 100 ml.)	2.64 ± 0.10 (12)	2.88 ± 0.07 (14)	1.999	>0.05
Alpha-one globulin (gm./100 ml.)	0.425 ± 0.018 (11)	0.401 ± 0.015 (14)	1.035	>0.2
Alpha-two globulin (gm./100 ml.)	0.678 ± 0.046 (11)	0.562 ± 0.020 (14)	2.507	<0.025
Beta globulin (gm./100 ml.)	0.900 ± 0.050 (12)	0.808 ± 0.044 (14)	1.392	<0.2
Gamma globulin (gm./100 ml.)	1.393 ± 0.104 (12)	1.166 ± 0.087 (14)	1.687	>0.1

TABLE 2

Serum glycoproteins of patients antepartum

Constituent	Mean ± S.E. (No. of observations) Diabetic	Normal	t	P
Hexose (mg./100 ml.)	126.6 ±4.2 (12)	123.1 ±4.7 (14)	0.171	>0.5
Hexosamine (mg./100 ml.)	94.2 ±4.2 (12)	88.7 ±3.9 (14)	0.957	<0.4
Sialic acid—Warren (mg./100 ml.)	91.4 ±3.3 (12)	84.9 ±2.4 (14)	1.637	>0.1
Sialic acid—Seibert et al. (mg./100 ml.)	119.9 ±7.0 (11)	118.2 ±4.3 (14)	0.204	>0.5
Total glycoprotein* as per cent of total protein	5.16 ±0.13 (12)	5.07 ±0.12 (14)	0.864	<0.4
Seromucoid hexose (mg./100 ml.)	12.1 ±0.8 (12)	15.9 ±1.1 (14)	2.715	>0.01

*Total glycoprotein = hexose + hexosamine + sialic acid (Warren).

The mean values for nonpregnant women were 106.5 ± 3.9 mg. per cent of hexose and 80.3 ± 2.9 mg. per cent of hexosamine. The mean value for seromucoid hexose was 9.8 ± 1.1 mg. per cent and became significantly elevated only in nondiabetic pregnant women.

Gestational age, birth weights and sex distribution of infants born to diabetic and nondiabetic mothers are graphically presented in figure 1. The average birth weight of an infant of a diabetic mother was 3,450 gm. in comparison to 3,230 gm. of a newborn of a nondiabetic mother. The data show the tendency to oversized infants which is well known to occur in offspring of diabetic mothers.[24,25]

A survey of individual values obtained by chemical analyses of cord blood revealed no difference in glycoprotein concentrations with respect to the age of the

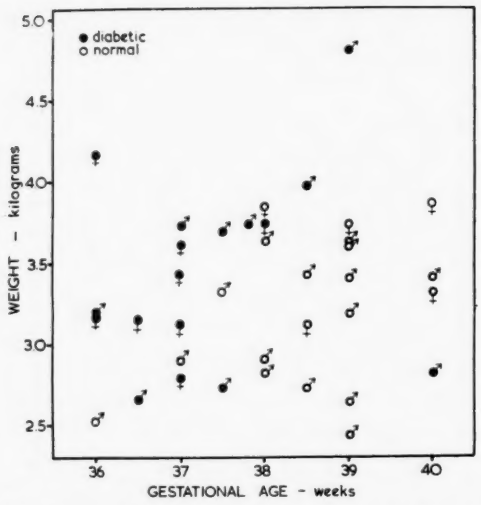


FIG. 1. Birth weights, gestational age and sex distribution in two groups of newborns. Infants of diabetic mothers reach the weight of infants of normal mothers at an earlier gestational age.

mother, route of delivery, number of previous pregnancies and gestational age within the limits encountered in our material. Thus, for statistical purposes the series of infants of diabetic and nondiabetic mothers were each considered as a homogeneous population.

The values for hematocrit, blood sugar, cholesterol and serum proteins found for the two groups of infants are recorded in table 3. The results of analyses of the serum protein-bound carbohydrate moiety are presented in table 4. The amounts of total hexose, hexosamine and sialic acid, determined by either method, were significantly elevated in infants of diabetic mothers. Curiously enough, the seromucoid hexose did not appreciably change. Neither did the alpha-one globulin fraction which contains much of the seromucoid, while the alpha-two and beta globulins were significantly elevated in infants of diabetic mothers.

Representative electrophoretic patterns of serum proteins stained for carbohydrate material are presented in figure 2. Serum specimens from infants of diabetic and normal mothers were chosen in a manner to cover the range of variations in glycoprotein concentrations found by chemical analysis. Although no attempt was made to evaluate patterns quantitatively, the results indicate that variations in the quantity of stainable glycoprotein were not limited to one electrophoretic fraction.

The test for C-reactive protein was negative in all infants.

DISCUSSION

The increased amounts of total protein-bound hexose, hexosamine and sialic acid found in blood of infants of diabetic mothers are by all available criteria a true meas-

TABLE 3

Blood chemistry of infants at birth

Constituent	Mean ± S.E. (No. of observations) Diabetic	Normal	t	P
Hematocrit (per cent formed elements)	57.0 ± 2.2 (14)	53.1 ±1.8 (17)	1.388	<0.2
Blood sugar (mg./100 ml.)	109 ±26 (14)	98 ±9 (17)	0.440	>0.5
Cholesterol (mg./100 ml.)	96 ± 7 (16)	102 ±6 (20)	0.632	>0.5
Total protein (gm./100 ml.)	5.19 ± 0.18 (17)	4.96 ±0.13 (20)	1.057	>0.2
Albumin (gm./100 ml.)	3.28 ± 0.14 (17)	3.32 ±0.08 (20)	0.257	>0.5
Alpha-one globulin (gm./100 ml.)	0.236 ± 0.019 (16)	0.220 ±0.013 (20)	0.710	>0.5
Alpha-two globulin (gm./100 ml.)	0.337 ± 0.024 (16)	0.284 ±0.012 (20)	2.101	<0.05
Beta globulin (gm./100 ml.)	0.520 ± 0.044 (17)	0.300 ±0.017 (20)	4.939	<0.001
Gamma globulin (gm./100 ml.)	0.836 ± 0.055 (17)	0.841 ±0.047 (20)	0.070	>0.5

TABLE 4

Serum glycoproteins of infants at birth

Constituent	Mean ± S.E. (No. of observations) Diabetic	Normal	t	P
Hexose (mg./100 ml.)	68.4 ±3.3 (16)	55.6 ±2.1 (20)	3.436	>0.001
Hexosamine (mg./100 ml.)	53.9 ±2.7 (16)	44.8 ±1.8 (20)	2.860	<0.01
Sialic acid—Warren (mg./100 ml.)	44.9 ±2.2 (17)	35.1 ±1.3 (20)	3.981	<0.001
Sialic acid—Seibert et al. (mg./100 ml.)	79.4 ±5.8 (16)	61.1 ±2.9 (20)	2.999	<0.005
Total glycoprotein* as per cent of total protein	3.29 ±0.19 (15)	2.73 ±0.06 (20)	3.174	<0.005
Seromucoid hexose (mg./100 ml.)	6.3 ±0.6 (16)	5.1 ±0.3 (18)	1.734	<0.1

*Total glycoprotein = hexose + hexosamine + sialic acid (Warren).

ure of an absolute elevation of the level of circulating glycoproteins. Although direct evidence is lacking, the increased alpha-two and beta globulin levels found in sera of these infants suggest that the rise in glycoproteins was not limited only to the carbohydrate moiety but concerned the protein moiety as well. The sum of the three carbohydrate constituents when calculated as per cent of total protein was significantly higher in infants of diabetic mothers as compared with their corresponding controls, and since there was no appreciable difference in the two groups with respect to hematocrit reading and total protein levels, hemoconcentration is eliminated as the sole reason for the rise in glycoproteins. Osler found a reduction of extracellular water, expressed as per cent of body weight, in infants of diabetic mothers which he attributed to the excessive deposition of fat and not to

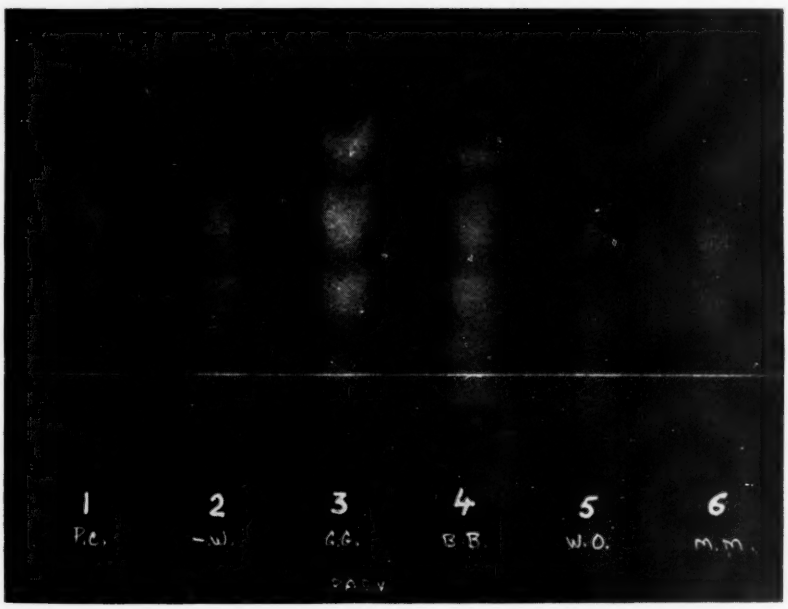


FIG. 2. Electrophoretic patterns of serum proteins stained for carbohydrate material. Infants of diabetic mothers: 1, 3, 4, 6. Infants of normal mothers: 2, 5. Note that the difference in dye uptake is not limited to one electrophoretic fraction.

dehydration of tissues.[40-43]

During intrauterine life the fetus of the diabetic women is exposed to blood sugar levels which are higher than normal and since glucose is an all-important precursor in the formation of the carbohydrate moiety of the glycoproteins,[44] it is perhaps not surprising to find higher serum levels in infants of diabetic mothers than in infants of nondiabetic mothers. There is no evidence as yet indicating that insulin is involved in the formation of the monosaccharidic components and their polymerization. Spiro has shown that glucosamine synthesis from glucose in the liver remained unimpaired in alloxanized rats in which glycogen synthesis was markedly depressed.[45] On the other hand, Schiller and Dorfman found the synthesis of acid mucopolysaccharides of the skin greatly inhibited in the same type of animals; treatment with insulin stimulated the synthesis of acid mucopolysaccharides and restored their metabolism close to normal.[46] This may be significant in the light of the work of Dorfman and Cifonelli[47] and other investigators like Bollet, Goodwin and Brown[48] who have shown that glucose is an essential precursor in the formation of mucopolysaccharides. The functional relationship between tissue mucopolysaccharides and serum glycoproteins is poorly understood and little is known about the origin, synthesis and the physiological significance of the latter. It is therefore difficult to give our observations a full interpretation.

The clinical histories were uneventful and it is unlikely that the infants of the diabetic mothers were suffering from an inflammatory process with appreciable tissue destruction. The negative C-reactive protein tests and the absence of elevated seromucoid levels tend to support this view.[38,49] The fact that the elevation of serum glycoproteins was observed in a group of infants with a tendency toward an accelerated rate of growth suggests that under these conditions the elevation was associated with new formation of tissues. It must be pointed out, however, that upon analysis of individual cases no close correlation was found between the concentration of the various carbohydrate components and birth weight. The weight range of infants born to normal as well as to diabetic mothers is wide. Since we have no information about turnover rates of the various glycoprotein fractions, the lack of a close correlation cannot be regarded as crucial. It is also logical to assume that the production and utilization of glycoproteins require a number of regulatory factors and not all of them can be related to fetal growth.

The results of our investigation tend to support the concept of a multiplicity of sites of origin for serum glycoproteins.[10] It may be recalled that the seromucoid hexose did not follow the rise encountered in the total carbohydrate moiety of infants of diabetic mothers. Neither did pregnancy in the diabetic and nondiabetic women affect the various components of the protein-bound carbohydrate moiety in a uniform manner. Although a rise in serum glycoproteins was encountered during pregnancy in normal women as well as in diabetic women and also in the offspring of diabetic mothers, there was enough difference in the concentration of the various carbohydrate components to suggest several sources of protein-bound carbohydrates. It is this multiplicity of origin which may perhaps account for the rise in glycoprotein levels under a variety of completely unrelated conditions. On the other hand, one cannot deny the possibility that a single source may produce a variety of glycoproteins in amounts sufficient to meet the requirements of the body.[18,44]

The knowledge of the chemistry of many glycoproteins is still scanty[50] and so long as there are no methods available for studying the origin and destination of chemically well defined entities, it will remain difficult to do more than speculate about the physiological significance of serum glycoproteins. Nevertheless, the finding of elevated glycoprotein levels in infants of a selected group of diabetic mothers whose metabolic condition during pregnancy and delivery was satisfactorily controlled seems to us of considerable importance. It may prove to be a valuable clue to some of the problems which are encountered in newborn infants of diabetic mothers.

SUMMARY AND CONCLUSIONS

Hexose, hexosamine and sialic acid contents of the carbohydrate moiety conjugated with serum proteins (glycoproteins) were determined in cord blood of seventeen infants of diabetic mothers and twenty infants of nondiabetic mothers. All three constituents were found to be significantly elevated in sera of infants of diabetic mothers.

The diabetic women selected for this study had no clinical signs of degenerative vascular lesions and showed no abnormal elevation of the same blood constituents when compared with normal pregnant women at the time of delivery. The average length of gestation was 37.3 weeks in the diabetic series (vaginal delivery or cesarean section) and 38.5 weeks in the normal series (all cesarean sections for pelvic disproportion).

The infants with elevated glycoprotein levels showed no evidence of any inflammatory process with tissue destruction (negative C-reaction protein test, normal sero-

mucoid levels and uneventful clinical histories). Therefore, it would appear that the increase in serum glycoproteins was associated with rapid growth of tissue. The fact that the elevation was found in a group of infants with a tendency to macrosomia tends to support this suggestion.

SUMMARIO IN INTERLINGUA

Glycoproteinas Seral in Neonatos ab Matres con Diabete

Le contento de hexosa, de hexosamina, e de acido sialic in le hydratos de carbon conjugate con proteinas seral (glycoproteinas) esseva determinate in le sanguine del cordon de dece-septe infantes de matres con diabete e de vinti infantes de matres sin diabete. Esseva constatate que le tres mentionate constituentes esseva significativemente elevate in le seros del infantes ab matres con diabete.

Le diabeticas seligite pro iste studio manifestava nulle signos clinic de degenerative morbo vascular e non habeva elevationes anormal del tres constituentes del sanguine in comparation con gravidas normal al tempore del parturition. Le duration medie del gestation esseva 37,3 septimanas in le serie diabetic e 38,5 septimanas in le serie normal. Le serie diabetic includeva parturitiones vaginal e sectiones cesaree. Le serie normal consisteva exclusivemente de casos de section cesaree a causa de disproportion pelvic.

Le infantes con elevate nivellos de glycoproteina monstrava nulle signo de un processo inflammatori comportante un destruction de tissu. Le test pro proteina C-reactive esseva negative, le nivello seromucoide esseva normal, e le curso clinic esseva sin incidente. Per consequente, il pare que le augmento del glycoproteinas del sero esseva associate con un rapide crescentia de tissu. Le facto que le elevation esseva trovate in un gruppo de infantes con un tendentia macrosomatic tende a reinfortiar ille conception.

ACKNOWLEDGMENT

The authors are indebted to Professor C. H. Best for his constant support and guidance in the course of this investigation. They are also indebted to staff physicians of the obstetrical departments of the Toronto General Hospital and the Women's College Hospital for the kind permission to include their patients as part of the material presented in this study. The authors wish to express their gratitude especially to Miss Y. Manus and Miss D. Muirhead for competent technical assistance and to Miss A. E. Spitzer, nurse-in-charge of the Labor Room of the Toronto General Hospital, for co-ordinating the collection of blood specimens.

This work was supported by a grant-in-aid from the Medical Research Council of Canada and in its initial phase by the J. P. Bickell Foundation of Toronto as well as by the Hoechst Pharmaceuticals of Canada.

REFERENCES

[1] Berkman, J., Rifkin, H., and Ross, G.: The serum polysaccharides in diabetic patients with and without degenerative vascular disease. J. Clin. Investigation *32*:415, 1953.

[2] Gilliland, I. C., Hanno, M. G., and Strudwick, J. I.: Protein-bound polysaccharides in diabetes with and without complications. Biochem. J., Lond., 56:xxxii, 1954.

[3] Keiding, N. R., and Tuller, E. F.: Protein-bound carbohydrate in the serum of diabetic patients with and without vascular complications. Diabetes *4*:37, 1955.

[4] Adlersberg, D., Wang, C. I., Rifkin, H., Berkman, J., Ross, G., and Weinstein, C.: Serum lipids and polysaccharides in diabetes mellitus. Diabetes *5*:116, 1956.

[5] Andreani, D. V., and Gray, C. H.: Serum polysaccharides in diabetes mellitus. Clin. Chim. Acta *1*:7, 1956.

[6] Ditzel, J., and Moinat, P.: The responses of the smaller blood vessels and the serum proteins in pregnant diabetic subjects. Diabetes 6:307, 1957.

[7] Mohnike, G.: Zur Pathophysiologie der diabetischen Angiopathie unter besonderer Berücksichtigung der eiweissgebundenen Zucker. Diabetes Mellitus, Proceedings of the 3rd Congress of the International Diabetes Federation, Stuttgart, G. Thieme Verlag, 1959, p. 70.

[8] Ejarque, P., Marble, A., and Tuller, E. F.: Proteins, lipoproteins and protein-bound carbohydrates in the serum of diabetic patients. Am. J. Med. *27*:221, 1959.

[9] Fry, I. K., Trounce, J. R., and Cook, C. A. G.: Vascular disease in diabetes mellitus. The relation of raised protein-bound hexose levels. Diabetes *8*:174, 1959.

[10] Winzler, R. J.: Determination of serum glycoproteins. Methods of Biochem. Analysis *2*:279, 1955.

[11] Shetlar, M. R., Foster, J. V., Kelly, K. H., Shetlar, C. L., Bryan, R. S., and Everett, M. R.: The serum polysaccharide level in malignancy and in other pathological conditions. Cancer Res. *9*:515, 1949.

[12] Shetlar, M. R., Kelly, K. H., Foster, J. V., Shetlar, C. L., and Everett, M. R.: Serum polysaccharide levels in pregnancy, parturition, and the postpartum state. Am. J. Obst. *59*:1140, 1950.

[13] Shetlar, M. R., Bullock, J. A., Shetlar, C. L., and Payne, R. W.: Comparison of C-reactive protein, glycoprotein and seromucoid in cancer, arthritis, tuberculosis and pregnancy. Proc. Soc. Exper. Biol., N. Y. *88*:107, 1955.

[14] Seibert, F. B., Seibert, M. V., Atno, A. J., and Campbell, H. W.: Variation in protein and polysaccharide content of sera in the chronic diseases, tuberculosis, sarcoidosis and carcinoma. J. Clin. Investigation *26*:90, 1947.

[15] Gersh, I., and Catchpole, H. R.: The nature of ground substance of connective tissue. Perspectives in Biology and Medicine 3:282, 1960.

[16] Heppelston, A. G., and Keyser, J. W.: The relationship of tissue formation and destruction to the levels of the serum protein-bound carbohydrate in rats bearing the Walker 256 carci-

noma. Brit. J. Exper. Path. *40*:263, 1959.

[17] Björnsjö, K. B., Werner, I., and Odin, L.: The influence of surgery on serum and urine hexosamine, serum mucoprotein, glutamic-oxalacetic transaminase (GOT) and C-reactive protein. Scand. J. Clin. and Lab. Invest. *11*:238, 1959.

[18] Werner, I.: On the regeneration of serum polysaccharide and serum proteins in normal and intoxicated rabbits. Acta Physiol. Scand. *19*:27, 1949.

[19] Weimer, H. E., Nishihara, H., and Ling, S. H.: Serial determinations of serum protein-bound carbohydrates and proteins during protein depletion and repletion in the adult rat. J. Nutrition *69*:151, 1959.

[20] Pedersen, K. O.: Fetuin: a new globulin isolated from serum. Nature, Lond. *154*:575, 1944.

[21] Deutsch, H. F.: Fetuin: the mucoprotein of fetal calf serum. J. Biol. Chem. *208*:669, 1954.

[22] Bergstrand, C. B., and Czar, B.: Protein-bound carbohydrates in human fetal serum. Scand. J. Clin. and Lab. Invest. *10*:379, 1958.

[23] Boas, N. F., and Peterman, A.: Effects of age, food intake and stress on plasma hexosamine levels in the rat. Proc. Soc. Exper. Biol., N.Y. *82*:19, 1953.

[24] Miller, H. C.: Offspring of diabetic and prediabetic mothers. Advances in Pediatrics *8*:137, 1956.

[25] Gellis, S. S., and Hsia, D. Y.: The infant of the diabetic mother. A.M.A. J. Dis. Child. *97*:1, 1959.

[26] Lustig, B., and Langer, A.: Über die Bestimmung und den Gehalt an freiem, Eiweiss- und gebundenem Nichteiweiss Zucker in normalen und pathologischen Serum, Liquor und Harn. Biochem. Zschr. *242*:320, 1931.

[27] Weimer, H. E., and Moshin, J. R.: Serum glycoprotein concentrations in experimental tuberculosis of guinea pigs. Am. Rev. Tuberc. *68*:594, 1953.

[28] Seibert, F. B., Pfaff, M. L., and Seibert, M. V.: A serum polysaccharide in tuberculosis and carcinoma. Arch. Biochem., N. Y. *18*:279, 1948.

[29] Warren, L.: The thiobarbituric acid assay of sialic acids. J. Biol. Chem. *234*:1971, 1959.

[30] Winzler, R. J., Devor, A. W., Mehl, J. W., and Smyth, I. M.: Studies on the mucoproteins of human plasma. I. Determination and isolation. J. Clin. Investigation *27*:609, 1948.

[31] Weichselbaum, T. E.: An accurate and rapid method for the determination of proteins in small amounts of blood serum and plasma. Am. J. Clin. Path. Tech. Sect. *16*:40, 1946.

[32] Kunkel, H. G.: Zone electrophoresis. Methods of Biochem. Analysis *1*:141, 1954.

[33] Björnsjö, K. B.: Staining of protein-bound serum polysaccharides in electrophoresis strips. Scand. J. Clin. and Lab. Invest. *7*:153, 1955.

[34] Bloor, W. R., Pelkan, K. F., and Allen, D. M.: Determination of fatty acids (and cholesterol) in small amounts of blood plasma. J. Biol. Chem. *52*:191, 1922.

[35] Anderson, H. C., and McCarthy, M.: Determination of C-reactive protein as measure of activity of disease process in acute rheumatic fever. Am. J. Med. *8*:445, 1950.

[36] King, E. J.: Micro-Analysis in Medical Biochemistry, London, J. & A. Churchill, 1951, 2nd ed., p. 23.

[37] Snedecor, G. W.: Statistical Methods, Ames, Iowa, The Iowa State College Press, 1956, 5th ed., p. 45.

[38] Rozansky, R., and Bercovici, B.: C-reactive protein during pregnancy and in cord blood. Proc. Soc. Exper. Biol., N. Y. *92*:4, 1956.

[39] Turumi, K. I., Hamagami, L. T., and Kenkel, H.: Relationship of sialic acid and C-reactive protein levels in human serum. J. A. M. A. *170*:2160, 1959.

[40] Osler, M.: Body water of newborn infants of diabetic mothers. Acta Endocrinol., Kbh. *34*:261, 1960.

[41] Osler, M.: Body fat of newborn infants of diabetic mothers. Acta Endocrinol., Kbh. *34*:277, 1960.

[42] Osler, M.: Renal function in newborn infants of diabetic mothers. Acta Endocrinol., Kbh. *34*:287, 1960.

[43] Osler, M.: Neonatal changes in body composition of infants born to diabetic mothers. Acta Endocrinol., Kbh. *34*:299, 1960.

[44] Spiro, R. G.: Studies on the biosynthesis of glucosamine in the intact rat. J. Biol. Chem. *234*:742, 1959.

[45] Spiro, R. G.: Role of insulin in two pathways of glucose metabolism: in vivo glucosamine and glycogen synthesis. Ann. N. Y. Acad. Sci. *82*:366, 1959.

[46] Schiller, S., and Dorfman, A.: The metabolism of mucopolysaccharides in animals. IV. The influence of insulin. J. Biol. Chem. *227*:625, 1957.

[47] Dorfman, A., and Cifonelli, J. A.: Biosynthesis of mucopolysaccharides: the uridine nucleotides of group A streptococci. Ciba Foundation Symposium on the Chemistry and Biology of Mucopolysaccharides. London, J. & A. Churchill, 1958, p. 64.

[48] Bollet, A. J., Goodwin, J. F., and Brown, A. K.: Metabolism of mucopolysaccharides in connective tissue. I. Studies of enzymes involved in glucuronide metabolism. J. Clin. Investigation *38*:451, 1959.

[49] Philipson, L., and Tveteras, E.: C-reactive protein in infancy, its appearance during the first year of life, transplacental passage and electrophoretic pattern. Acta. Paediat., Upps., *46*: 1, 1957.

[50] Winzler, R. J.: Glycoproteins. The Plasma Proteins. Edited by Putnam, F. W., New York and London, Academic Press, 1960, volume 1, p. 309.

Carbohydrate Metabolism in Pregnancy

IV. Studies on the Permeability of the Rat Placenta to I^{131} Insulin

Charles J. Goodner, M.D., and Norbert Freinkel, M.D., Boston

The contributions of placental mechanisms for insulin disposition to gestational changes in carbohydrate metabolism have been under continuing investigation in this laboratory.[1-3] In the course of these studies, it was noted that relatively small quantities of radioactivity appear in the fetal rat following the administration of I^{131}-insulin to the mother.[3] Accordingly, the present experiments were performed to characterize transplacental transport of insulin more precisely. Evidence has been collected which indicates that the passage of insulin across the rat placenta near term is extremely limited and that the placenta constitutes a relatively impermeable barrier to insulin flux.

METHODS

1. *Materials*: Sterile injection solutions of crystalline I^{131}-labeled bovine insulin* were prepared as described previously and supplemented with fresh rat plasma to minimize glassware adsorption and spontaneous degradation.[1] Experimental animals consisted of pregnant albino rats (Sprague-Dawley descendants, Charles River Breeding Laboratories) which had been mated twenty to twenty-one days earlier during a controlled twelve-hour period. They were allowed free access to water and Purina pellets prior to study.

2. *Experimental Procedures*: Animals were anesthetized with sodium pentobarbital and maintained under light surgical anesthesia until sacrifice. Solutions of labeled insulin were injected into exposed saphenous veins of anesthetized rats by either (a) single injection, or (b) continuous infusion.

A. *Single Injection Studies*—Animals were pretreated with 50 mg. glucose per 100 gm. body weight intraperitoneally to prevent hypoglycemia. Thereafter, 0.16 units of labeled insulin per 100 gm. body weight (1.0 to 1.5 ml.) was injected intravenously in thirty seconds or less. Rats were sacrificed twenty to forty minutes later. The abdomen was opened at the midline and the animals were exsanguinated from the abdominal aorta into an heparinized syringe. The uterus was excised, blotted, and quickly weighed to the nearest 0.1 gm. and opened along the antimesenteric margin. Aliquots of amniotic fluid were collected from bulging amniotic sacs with a No. 26 needle and syringe. The fetuses and placentas were delivered and blotted briefly to remove any adherent maternal blood. Several fetuses were rapidly frozen by placing them in liquid nitrogen or in powdered solid carbon dioxide. Representative placentas and portions of maternal liver were similarly frozen. The remaining fetuses were observed for motility and then bled from the incised neck.

B. *Continuous Infusion Studies*—Constant infusion was employed to achieve prolonged equilibrium and to minimize the analytical limitations posed by the rapid degradation of insulin in the pregnant rat.[3] Priming injections of glucose and labeled insulin were given in quantities designed to achieve varying degrees of hyperglycemia and a total of 0.1 unit I^{131}-insulin in the extrapancreatic "insulin space." The calculations were based on estimated volumes for the distribution of glucose[4] and insulin[5] of 30 per cent of the body weight. Immediately thereafter, sustaining infusions of glucose and insulin (3.0 to 5.0 ml./hour in different experiments) were started with a motor-driven syringe pump in quantities designed to maintain constant blood levels of these moieties. The infusion apparatus was calibrated both volumetrically and by radio-assay of timed samples before and after each experiment. The calibration was checked by periodic direct measurement of the volume delivered during the course of each infusion. Throughout the infusion period, serial samples of maternal blood were collected from the tail to document equilibrium conditions. Animals were sacrificed after one or three hours of continuous infusion and processed immediately, as in the *single injection* experiments.

3. *Analytical Technics*: Buffer-flow chromatography as previously described[3,5] was employed for identification

* I^{131}-labeled crystalline beef insulin was purchased from Abbott Laboratories, Oak Ridge, Tennessee, with an initial specific activity of 4 to 7 mc./mg.

From the Thorndike Memorial Laboratory and Second and Fourth (Harvard) Medical Services, Boston City Hospital, and the Department of Medicine, Harvard Medical School, Boston.

and quantitation of the tracer amounts of I^{131}-insulin in plasma, amniotic fluid and tissue extracts. In this technic, intact labeled insulin remains adsorbed at the site of application by virtue of its affinity for filter paper whereas radioactive degradation products are carried beyond the origin by hydrodynamic flow. Thus, soluble radioactivity is chromatographically resolved into distinct zones corresponding to intact insulin ("origin" radioactivity) and products of insulin breakdown ("mobile" radioactivity). To enhance the sensitivity of the procedure, it was modified for analysis of large aliquots. Replicate 0.020 ml. portions of fluid were applied in a single line across the width of a sheet of filter paper so that 0.2-0.4 ml. specimens could be chromatographed at one time. Less than 0.2 ml. was not analyzed in any instance. The large sheets were subsequently divided into 1 cm. segments paralleling the line of applications and the individual segments were rolled into cylinders and counted in a well-type scintillation detector. Preliminary studies indicated that fetal plasma components did not modify the adsorption of intact I^{131}-insulin to filter paper thereby validating the use of buffer-flow chromatography for characterization of insulin in fetal fluids.

Specific steps were instituted to minimize the likelihood of insulin degradation in vitro prior to chromatography. Control experiments indicated that significant catabolism of insulin occurred during incubation of I^{131}-insulin (10 to 10,000 microunits/ml.)* in maternal or fetal blood at 37° (figure 1). The process was especially pronounced in fetal blood which had been collected as described above. However, such degradation did not occur at 0° C. (figure 1). Therefore, in all studies, fetal and maternal blood samples were collected into chilled tubes, processed at 0°-4° C., and chromatographed within less than four hours following collection. Further to minimize the degradatory potential of any insulinase which might have been leached from cut tissue surfaces[6,7] modified immunoelectrophoresis was performed in a few studies. Maternal and fetal blood specimens were collected directly into chilled tubes containing measured quantities of human serum rich in insulin-binding antibody.[5] The radioactivity in 0.2-0.4 ml. aliquots of the resulting mixtures was characterized by combined chromatography and electrophoresis.[6] Herein, "free" insulin (i.e., insulin which is neither degraded nor complexed to antibody) remains adsorbed to the "origin" while the radioactivity which moves beyond the site of application during chromatography is partitioned by subsequent electrophoresis into "antibody-bound" insulin ("gamma-beta globulin radioactivity") and insulin degradation products ("alpha-globulin-albumin radioactivity"). Control studies documented that such complexing of insulin to antibody effectively protected the labeled hormone from the action of fetal "insulinase"[2] in vitro (figure 2). The manifest degradation of I^{131}-insulin which fetal homogenates produced at 0° C. could be arrested within five minutes following the introduction of antibody-rich serum (figure 2).

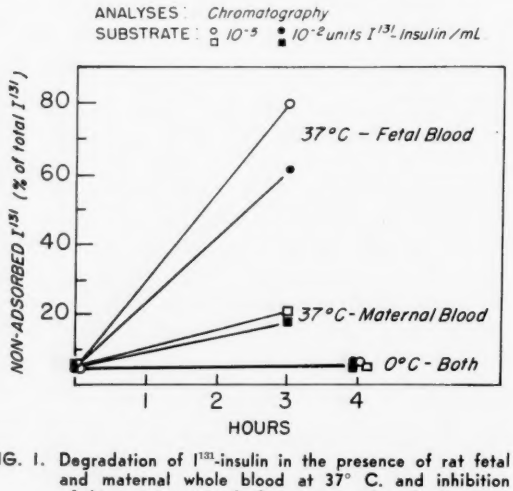


FIG. 1. Degradation of I^{131}-insulin in the presence of rat fetal and maternal whole blood at 37° C. and inhibition of this activity at 0° C. Quantitation of insulin degradation was based upon chromatographic analysis of the reaction mixtures.

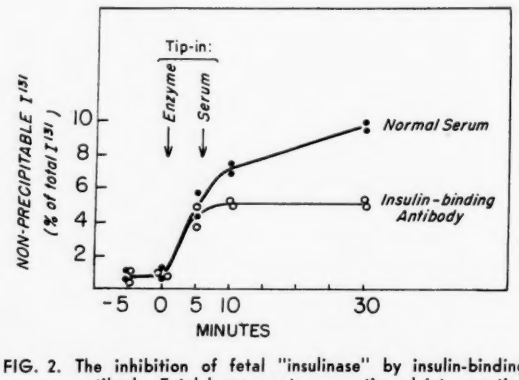


FIG. 2. The inhibition of fetal "insulinase" by insulin-binding antibody. Fetal homogenates were tipped into reaction mixtures containing I^{131}-insulin. Five minutes, thereafter, normal human serum or human serum rich in insulin-binding antibody was introduced. Degradation of insulin was assessed by the liberation of trichloracetic acid-soluble radioactivity. The incubation was performed at 0° C.

*One microunit (μU) equals .000001 unit insulin.

For analysis of tissue radioactivity, acid-alcohol extracts were prepared. Frozen fetuses, placentas or maternal livers were homogenized in Potter-Elvehjem homogenizers with two volumes of chilled 0.1 N HCl in 5 per cent ethyl alcohol. Homogenates were centrifuged for thirty minutes at 4° C. and 15,000 x g, and the insoluble residues were re-extracted a second time with two volumes of acid-alcohol. Soluble radioactivity in the pooled supernatant fluids was resolved by buffer-flow chromatography as described above. Insoluble tissue residues were dissolved in alkali and assayed for total nonextractable radioactivity. Control recovery experiments were performed by directly adding intact I^{131}-insulin to frozen fetuses or maternal liver prior to homogenization (1,000 microunits of insulin/gm. tissue). With fetuses, 40 to 50 per cent of the added counts remained in the insoluble residue whereas all but 10 to 30 per cent could be recovered from maternal liver by two extractions with acid-alcohol. For both tissues, 70 to 80 per cent of the radioactivity in the acid-alcohol extracts was adsorbed to the "origin" during buffer-flow chromatography. Thus, net recovery of added I^{131}-insulin as chromatographically-intact material ranged from 35 to 50 per cent for fetal tissues and 50 to 70 per cent for maternal liver.

Methods for radioactive assay of I^{131}-insulin in this laboratory have been described elsewhere.[1-3] Glucose in maternal and fetal plasma was estimated with glucose oxidase[8] following precipitation of plasma proteins with $BaSO_4$-$Zn(OH)_2$.[9]

RESULTS

1. *Distribution of I^{131}-Insulin Following Single Intravenous Injections*:

Values for radioactive assay of tissues and plasmas from six twenty-one-day pregnant rats are listed in table 1. Animals averaged 300 gm. in weight and had received 0.48 units of I^{131}-insulin (i.e., 1.1 x 10^8 cpm) by intravenous injection twenty or forty minutes prior to sacrifice. Total radioactivity in maternal plasma averaged 943,760 and 932,450 cpm/ml. respectively at these intervals. During chromatographic resolution by buffer-flow chromatography, an average of 41.9 and 14.8 per cent of the total radioactivity in the maternal plasma remained adsorbed at the "origin" (figure 3). Thus, on the basis of the specific activity of the administered labeled hormone, maternal circulating I^{131}-insulin at twenty and forty minutes averaged 1,829 and 634 microunits (μU) per ml. plasma. Contrariwise, no such distinct aggregation of radioactivity at the "origin" was observed during chromatography of fetal plasma. Despite the presence of an average of 63,179 and 211,303 cpm/ml. in the respective fetal specimens obtained at twenty and forty minutes, the radioactivity which remained at the site of application during chromatography of 0.2 ml. aliquots barely exceeded background (figure 3). Furthermore, in the subsequent segments of the fetal chromatograms (i.e., beyond the site of application), the radioactivity did not return to base-line levels. Instead, it progressively increased to merge abruptly with the sharp peak of counts overlying the plasma proteins and coinciding with the migration of the "mobile radioactivity" (i.e., the products of insulin catabolism) (figure 3). Presumably, the rising albeit extremely low level of radioactivity which extended from "origin" to solvent front did not represent intact I^{131}-insulin but rather constituted chromatographic "trailing" of labeled degradation products. It did not exceed 0.8 per cent of total radioactivity in any speci-

TABLE 1

Administration of I^{131}-insulin by single intravenous injection to twenty-one-day pregnant rats

		Plasma radioactivity† (cpm/ml.)				Tissue radioactivity† (cpm/gm.)					
	Time*	Maternal		Fetal		Maternal liver		Placenta		Fetus	
No.	(min.)	Total	"Origin"‡	Total	"Origin"	Total	"Origin"	Total	"Origin"	Total	"Origin"
1	20	1,174,450	516,000	65,520	330	764,000	368,000	376,100	54,500	67,500	380
2	20	985,000	374,000	48,860	220	1,105,000	268,000	311,000	54,400	42,000	94
3	20	671,830	295,000	75,210	307	635,000	157,000	322,000	34,400	45,000	157
4	40	874,225	131,000	195,910	475	595,000	59,300	477,000	31,400	161,000	1,200
5	40	894,925	143,000	199,800	270	336,000	69,100	574,000	34,200	278,000	1,500
6	40	1,028,200	137,000	238,200	435	508,000	46,800	556,000	38,900	211,000	1,350

*Animals injected with I^{131}-insulin in single administration and sacrificed twenty or forty minutes thereafter. Values above represent findings at sacrifice. I^{131}-insulin in all experiments was adjusted to contain 216 cpm per microunit.

†Radioactivity in counts per minute (cpm) was resolved by buffer-flow chromatography. Total fluid specimen applied for chromatography consisted of at least 0.2 ml. in each instance.

‡"Origin" denotes the portion of the total radioactivity which remained adsorbed at the site of application and constitutes a maximum estimate for undegraded I^{131}-insulin. Values for tissue extracts were corrected for estimated lossses during the recovery procedure. See text for further characterization of "origin" material in fetal plasma or extracts.

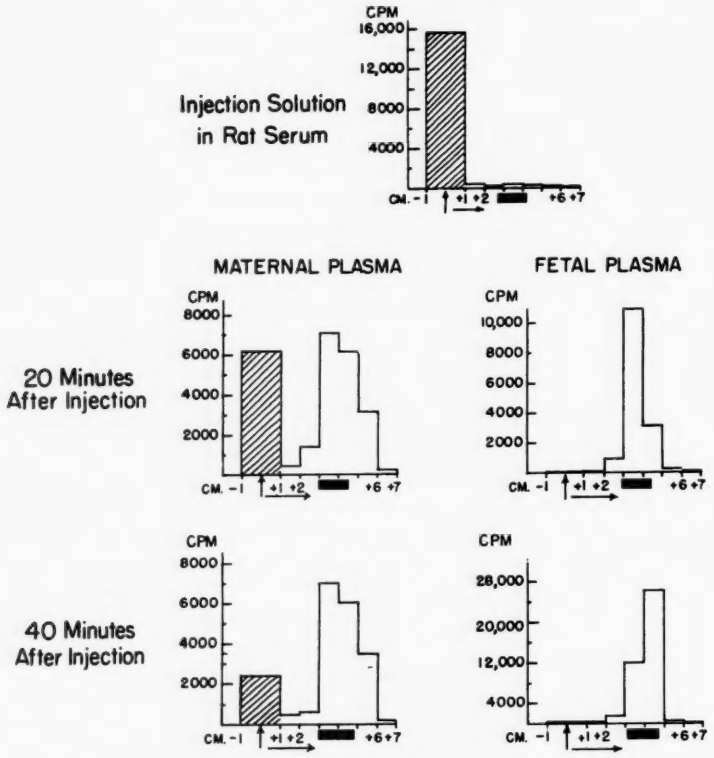


FIG. 3. Chromatographic analysis of radioactivity present in maternal and fetal plasma after the administration of I^{131}-insulin by single intravenous injection into the maternal circulation. Maternal and fetal plasma were obtained from rats sacrificed at twenty and forty minutes after injection. The vertical arrows denote sites of application and the horizontal arrows denote direction of chromatographic migration. Radioactivity remaining at the site of application during chromatography, i.e., "origin" radioactivity (intact I^{131}-insulin) is depicted by the shaded columns. The open columns denote "mobile" radioactivity (i.e., degradation products of I^{131}-insulin). Migration from the "origin" during buffer-flow chromatography in centimeters (cm.) is indicated on the horizontal axis. For radioactive assay, one cm. segments were counted. Counts observed in individual segments (cpm) are shown by the height of the bars. The injection solution chromatographed simultaneously contained 94 per cent "origin" radioactivity.

men and varied in absolute magnitude with the total number of counts applied to the chromatogram. In part, the phenomenon could be reproduced by chromatographing the trichloracetic acid-soluble radioactivity which is liberated during proteolysis of I^{131}-insulin in vitro by purified "insulinase" preparations. Nonetheless, in order to assign an *upper* limit to the amount of radioactivity that *might* represent fetal I^{131}-insulin, and to define the *lower* analytical limits of the assay procedure, *all* of these poorly resolved counts at the "origin" of fetal chromatograms were converted to microunits of insulin.* By this convention, fetal plasma did not contain more than 1.32 and 1.82 μU I^{131}-insulin/ml. twenty and forty minutes following injection of the mother, and average gradients for I^{131}-insulin between maternal and fetal plasma at these intervals were at least 1,386:1 and 348:1.

Analysis of amniotic fluid and acid-alcohol extracts of fetal tissues disclosed that the absence of circulating I^{131}-insulin in the fetus was not due to extravascular pooling (table 2, figure 4). Despite the presence of appreciable total radioactivity in both amniotic fluid and fetal extracts, neither contained counts which remained adsorbed at the "origin" as distinct peaks during chromatography. For fetal extracts, the "trailed" radioactivity at the "origin" (figure 4) at most represented 0.97 and 6.25 μU I^{131}-insulin/gm. of tissue at twenty minutes and forty minutes respectively. The maximum values for amniotic fluid were even less. On the other hand, clearly defined and sharply-demarcated peaks of "origin" radioactivity were observed in chromatograms of acid-alcohol extracts of maternal liver and placenta (figure 4). During the twenty- to forty-minute interval, the average concentration of "origin" radioactivity in placental extracts declined only 27 per cent whereas corresponding values in maternal plasma and maternal liver decreased by 65 and 78 per cent respectively (table 1). The relative preservation of "origin" radioactivity

*For such calculations, the two or three segments of fetal chromatograms were employed which corresponded to area of peak "origin" radioactivity in concurrently chromatographed specimens of maternal plasma. Values for extracts of fetal tissue were expressed per gram of fetus and further corrected for losses due to incomplete recovery of radioactivity in the acid-alcohol extraction procedure.

TABLE 2

Administration of I^{131}-insulin by continuous infusion to twenty-one-day pregnant rats

No.	Time* (min.)	Plasma glucose (mg. per 100 ml.) Maternal	Plasma glucose (mg. per 100 ml.) Fetal	Plasma radioactivity† (cpm/ml.) Maternal Total	Plasma radioactivity† (cpm/ml.) Maternal "Origin"	Plasma radioactivity† (cpm/ml.) Fetal Total	Plasma radioactivity† (cpm/ml.) Fetal "Origin"	Placental radioactivity† (cpm/gm.) Total	Placental radioactivity† (cpm/gm.) "Origin"
1	180	194	109	832,000	200,000	332,000‡	375		
			126			243,000	750		
2	180	107	61	790,000	263,000	258,000	1,000		
3	60	473	280	1,540,000	508,000	222,000	510	1,110,000‡	89,000
			261			203,000	350	983,000	88,000
								765,000	76,000
4	180	399	245	1,230,000	492,000	378,000	1,300	1,200,000	144,000
			217			370,000	625	1,500,000	180,000
								1,200,000	132,000

*Animals infused at constant rate for 60 or 180 minutes with I^{131}-insulin in glucose-containing solution. Values above represent findings at sacrifice. I^{131}-insulin was labeled with 154 cpm per microunit in Experiment 1; 147 cpm/uU in Experiment 2; and 216 cpm/uU in Experiments 3 and 4.

‡Radioactivity in counts per minute (cpm) chromatographically resolved as in Table 1.

‡Multiple values represent assays from separate fetuses or placentas.

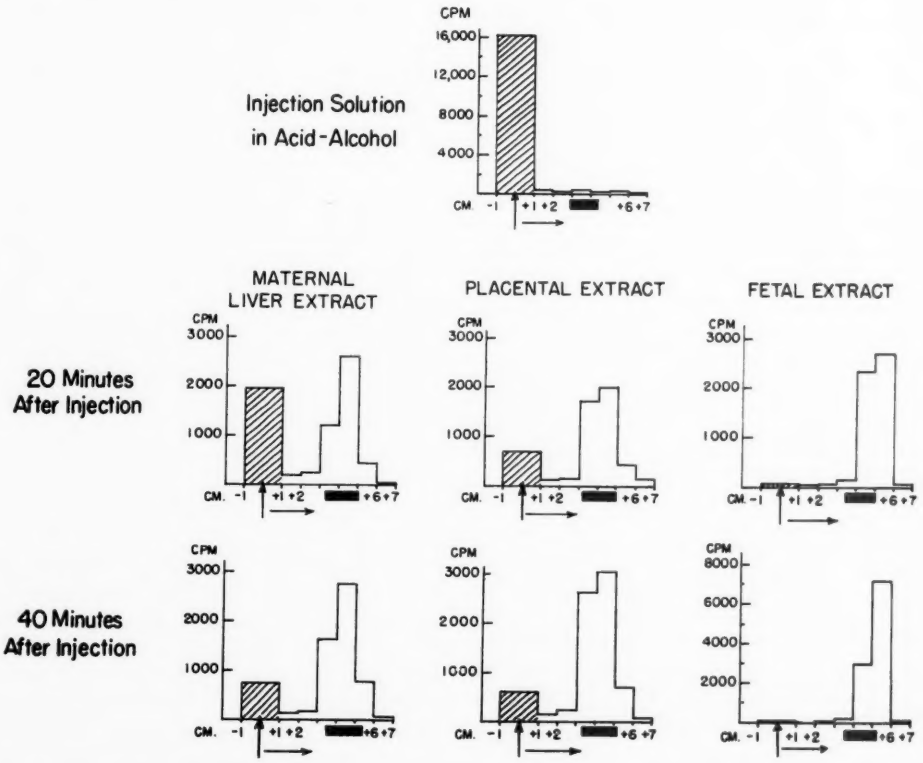


FIG. 4. Chromatographic analysis of radioactivity (cpm/paper segment) present in acid alcohol extracts of maternal liver, placenta and fetal tissue after single intravenous injection of I^{131}-insulin into the maternal circulation (plotted as in figure 3). Tissues were collected from animals sacrificed at twenty and forty minutes after injection. The injected I^{131}-insulin chromatographed after similar extraction contained 95 per cent "origin" radioactivity.

in placental extracts would tend to exclude occluded blood as the basis for all of the manifest placental "insulin." Rather, it would suggest that accumulation of insulin within the placenta exceeded the intraplacental rate of insulin degradation, and would be compatible with intraplacental sequestration of intact hormone.

2. *Distribution of I^{131}-Insulin Following Continuous Intravenous Infusion*:

Values for maternal plasma glucose, total radioactivity, and "origin" radioactivity observed during the course of a representative three-hour infusion of I^{131}-insulin in glucose-containing media are depicted in figure 5. Maternal blood sugar remained at constant, mildly hyperglycemic levels from sixty minutes onward. Concomitantly, the level of total circulating radioactivity rose whereas the concentration of counts exhibiting the chromatographic characteristics of intact insulin (i.e., "origin" radioactivity) remained constant. The constancy of plasma glucose and I^{131}-insulin would indicate that prolonged distribution equilibrium was achieved by infusion technics.[4]

Maternal and fetal values observed at sacrifice in the four infusion experiments are summarized in table 2. Levels of glucose in maternal plasma were uniformly nearly twice as great as the simultaneous concentrations of glucose in fetal plasma. Values for plasma glucose in duplicate fetuses from a single litter were in close agreement. Total radioactivity in duplicate fetal plasma samples was consistently lower at sacrifice than in the corresponding maternal plasma. In the three-hour infusion experiments, gradients for total I^{131} between maternal and fetal plasma averaged 3:1. Chromatographic analysis of 0.2 ml. aliquots of fetal plasma failed to demonstrate "origin" radioactivity in excess of the trailing phenomenon described above despite the fact that higher concentrations of total circulating radioactivity were achieved and sustained by the infusion

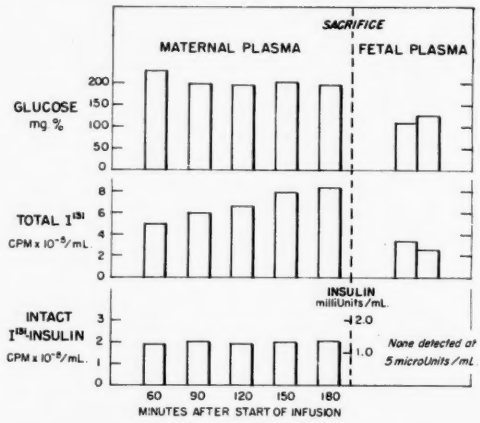


FIG. 5. Maternal plasma values during constant intravenous infusion into the maternal circulation. The heights of the bars denote: (a) plasma glucose in mg. per 100 ml.; (b) total radioactivity in I^{131} cpm/ml.; and (c) intact I^{131}-insulin ("origin" radioactivity) in cpm/ml. and milliunits/ml. Fetal plasma values at sacrifice represent analyses from two fetuses.

technic (table 2, figure 6). On the basis of the specific activities of the labeled hormone preparations, this ill-defined radioactivity at the site of application in chromatograms of fetal plasma could not have represented more than 6.80 μU of I^{131}-insulin/ml. in any experiment whereas concurrent maternal values ranged from 1,299 to 2,352 μU/ml.

In two of the three-hour infusion experiments, duplicate specimens of fetal and maternal blood were also collected directly into chilled tubes containing human serum rich in insulin-binding antibody. The resultant mixtures of human serum and rat plasma were analyzed by combined chromatography and electrophoresis. Figure 7 illustrates one such analysis and is representative of the findings with all of the samples. Three peaks of radioactivity were present in the maternal plasma representing: (a) "origin" radioactivity, (b) antibody-bound insulin, and (c) insulin degradation products. In contrast, fetal plasma exhibited significant radioactivity only in the region corresponding to degradation products.

Amniotic fluid contained less total counts per milliliter than fetal plasma and exhibited negligible "origin" radioactivity during chromatography (figure 6). Similar negative findings were obtained with acid-alcohol extracts of the fetuses (figure 6). Contrariwise, the acid-alcohol extracts of placentas which were prepared in Experiments 3 and 4 (table 2) contained abundant "origin" radioactivity. For these studies, twenty-one-day

*The manifest attainment of distribution equilibrium by constant infusion technics permits application of the method of Schwartz[10] for calculating the virtual volume of I^{131}-insulin distribution. In this technic, the distribution volume of a rapidly disappearing moiety may be estimated by dividing its total clearance (as assessed during equilibrium infusion) by its rate of removal from the circulation following distribution equilibrium. Using the values for total clearance of I^{131}-insulin which we obtained during the present infusion studies, and the average value for fractional I^{131}-insulin turnover in the twenty-one-day pregnant rat which we previously determined by single injection methods,[3] "insulin spaces" of 22.9 per cent, 20.9 per cent, 17.3 per cent and 16.0 per cent of body weight could be derived for the respective four animals of infusion experiments 1-4 (table 2).

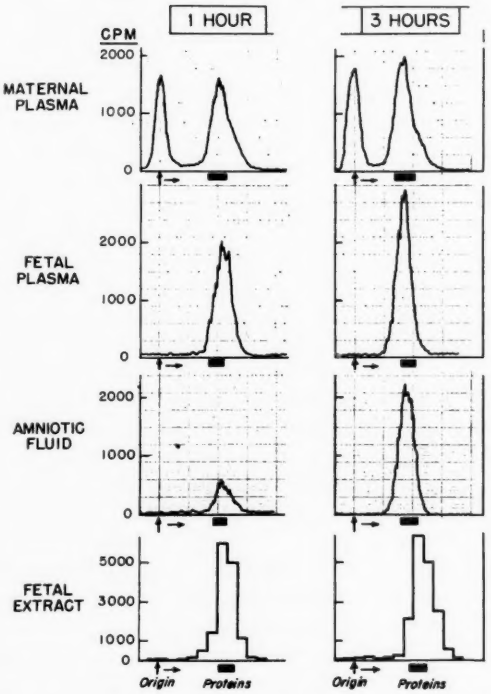


FIG. 6. Chromatographic analysis of the radioactivity present in maternal plasma, fetal plasma, amniotic fluid and acid-alcohol extracts of fetus after one and three hours of constant infusion of I^{131}-insulin into the maternal circulation. Individual segments of chromatograms were counted and the findings for fetal extracts (plotted as in figures 3 and 4) are depicted above. In addition, the chromatograms of maternal plasma, fetal plasma and amniotic fluid were also assayed by passage through a calibrated, automatically-recording strip scanner (Nuclear, Chicago). Results of such analyses are shown in the above illustration.

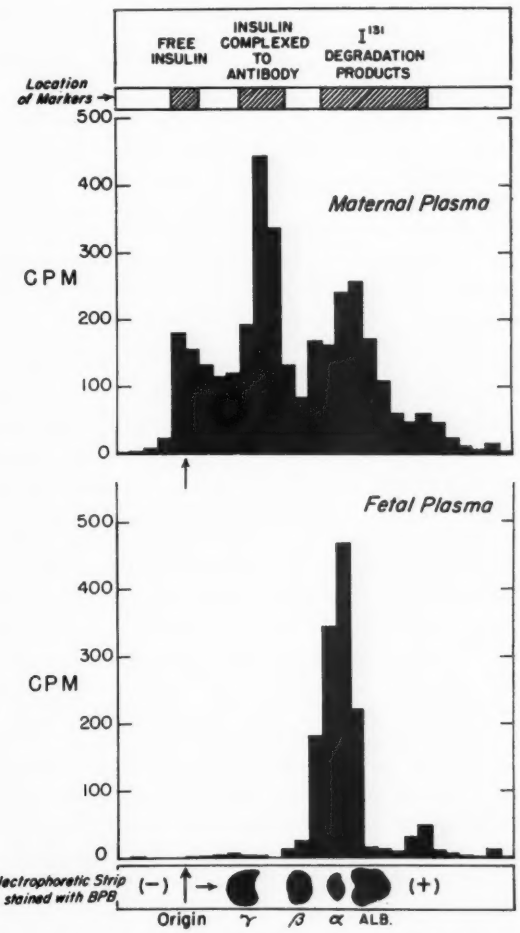


FIG. 7. The use of combined chromatography and electrophoresis to resolve radioactivity in maternal and fetal plasmas which had been collected into sera containing insulin-binding antibody. Maternal and fetal specimens were obtained after three hours of constant infusion into the maternal circulation. Staining with bromphenol blue (BPB) was employed to localize plasma proteins.

pregnant rats of matched weight were infused at identical rates with the same injection solution. Although similar maternal plasma levels of I^{131}-insulin were achieved in both animals, the intact I^{131}-insulin in the placenta following three hours of infusion (Experiment 4, table 2) was significantly greater than that observed after one hour of infusion (Experiment 3, table 2). As in the single injection experiments, this latter phenomenon could be in accord with progressive intraplacental accumulation of labeled hormone but the possibility that it merely represents delayed equilibration with placental extravascular extracellular fluid cannot be excluded.

DISCUSSION

Previous attempts to assess transplacental passage of insulin have been based largely on indirect technic. Insulin has been injected into the maternal or fetal circulation and subsequent changes in blood sugar on the uninjected side have been interpreted as evidence for insulin flux across the placenta.[11–17] That such alterations need not denote insulin passage and may only reflect a re-equilibration of glucose across the placenta following induction of hypoglycemia on the injected side was first suggested by Olow.[12] To circumvent this objection, Knobil and Josimovich[18] introduced insulin or control solutions directly into the uterine arteries of opposite uterine horns of pregnant rats, and measured serial alterations in the blood sugar of fetuses from the two

horns. In this manner, they demonstrated that quantities of insulin which were insufficient to affect maternal blood sugar (i.e., 0.05 units in 0.1 ml. saline) produced delayed fetal hypoglycemia whereas comparable effects did not result from the injection of 0.1 ml. saline into the other uterine horn. They concluded that there is transplacental passage of insulin in the twenty-one-day pregnant rat. However, other explanations might equally obtain if one includes the possibility of intraplacental effects in analysis of their findings. For example, the intra-arterial injections per se could have altered placental permeability. Although Knobil and Josimovich attempted to reduce arterial spasm by "topical application of 5 per cent procaine hydrochloride," the fact that blood sugars in all fetuses initially rose to approach and even exceed maternal levels would be compatible with such placental changes especially since the low levels of glucose-6-phosphatase activity in fetal rat liver at this stage of gestation[19,20] would preclude appreciable entry of glucose from fetal sources. Alternatively, if insulin were to influence glucose secretion in the placenta, as in the liver,[21] the late reduction in fetal blood sugar might reflect a direct effect of insulin upon this intraplacental process. As yet there are no data relevant to a direct action of insulin upon the rat placenta. However, insulin does act upon the human placenta,[22] and it is tempting to ascribe to some intraplacental regulation of glucose transfer the higher maternal than fetal blood sugar values which have been reported by others in humans[23–26] and rats,[15,18] and which were observed in the present studies during conjoint infusions of glucose and insulin into the mother. Clearly, conjectures regarding the role of insulin must await elucidation of placental glucose transport in normal as well as diabetic animals. However, Hagerman and Wellington's finding of augmented glucose production in vitro by placentas from alloxan-diabetic rats[27] are compatible with the possibility of insulin participation, and lend credence to the suggestion that changes in fetal blood sugar could result from direct effects of insulin upon the placenta.

In the present experiments, I^{131}-insulin was employed to examine directly the question of transplacental insulin exchange in the rat. Various methodological refinements were instituted to enhance analytical accuracy and specificity. Special precautions such as chilling, stabilization with insulin-binding antibody, acid-alcohol extraction and rapid chromatographic analysis were taken to obviate the likelihood of insulin-degradation in vitro during collection of specimens from sources rich in "insulinase." For actual analysis, a tenfold increase in the sensitivity of buffer-flow chromatography was achieved by modifying the procedure to permit analysis of 0.2 ml. rather than 0.020 ml. aliquots. Moreover, chromatograms were counted as multiple, separate segments to differentiate between "trailing" phenomena and discrete chromatographic entities. Finally, in a few studies, maximal specificity was achieved by interacting fetal specimens with insulin-binding antibody and combining chromatography with electrophoresis.

By all of these modalities, clearly defined radioactivity exhibiting the chromatographic or immunologic characteristics of intact I^{131}-insulin could not be demonstrated in any specimen of fetal origin. Several factors besides placental impermeability could account for the apparent negative findings. First, it is conceivable that insulin does cross the placenta but is *immediately* degraded within the fetus. The fact that rat fetal size precludes the collection of anything save mixed arterial and venous samples would tend to exaggerate this phenomenon in any fetal specimen. However, it should be noted that total radioactivity (i.e., intact plus degraded insulin) in fetal plasma was uniformly less than the maternal value. Moreover, the likelihood of "instantaneous degradation" of I^{131}-insulin is rendered less tenable by control studies wherein labeled hormone was directly injected into fetuses in vitro. When such fetuses were removed at serial intervals within five to thirty minutes following insulin instillation, 60 to 20 per cent of the radoactivity in acid-alcohol extracts exhibited the chromatographic characteristics of intact insulin. Second, if transplacental transport of insulin were slow, and if placental perfusion were partly rate-limiting, then the rapidity of insulin turnover in the maternal circulation[3] might preclude significant delivery of insulin to the placenta during a finite interval although integrated delivery over a prolonged period might be appreciable. This constitutes a valid objection to single injection studies. However, it cannot be applied to the continuous infusion experiments. Herein, as documented by multiple analyses of maternal blood, the placenta was perfused by constant levels of I^{131}-insulin for more than two hours. Indeed, the very fact that maternal levels of I^{131}-insulin remained constant would militate against appreciable penetration into a slowly equilibrating subdivision of the "insulin space." Finally, it is possible that there exists a transport maximum for the transplacental passage of insulin. This maximum could have been exceeded by the combined impact of native insulin plus exogenous labeled hormone. In the absence of data for normal levels of plasma insulin in gravid rats, one cannot evaluate the extent to which physiological concentrations may have been exceeded in the present studies. However, in any event,

one might anticipate that some labeled hormone should be demonstrable in the fetal circulation and that this moiety might change in association with alterations in maternal levels of I^{131}-insulin (as in the single injection studies). As calculated in the Results section, even if all of the "origin" radioactivity in fetal specimens is designated as intact insulin, values greater than 7 microunits I^{131}-insulin per ml. or gm. were not observed with any preparation and gradients for labeled hormones between maternal and fetal plasma uniformly exceeded 260:1. Thus, although possible transplacental passage of a limited quantity of insulin cannot be wholly excluded, and the present observations do not justify conclusions regarding earlier phases of rat pregnancy, it is apparent that the placenta of the twenty-one-day pregnant rat is relatively impermeable to insulin. Similar findings have been reported by Roberts, Buse and Buse in humans.[28] Using trichloracetic acid (TCA) precipitation of umbilical blood and administering I^{131}-insulin seven minutes to four hours prior to delivery, they were unable to demonstrate "significant" transfer of insulin across the placenta.* On the other hand, Josimovich and Knobil with an elegant experimental system and chromatographic analyses of plasma observed definite transplacental passage of I^{131}-insulin in the 107 to 129-day pregnant monkey.[30] However, even in this species, large concentration gradients for insulin between maternal and umbilical venous plasma consistently obtained and the physiological adequacy of the fetal delivery could not be assessed.[30]

From all of the foregoing, it is apparent that the placenta constitutes a substantial barrier to the free flux of insulin from mother to fetus. Not only does the placenta contain mechanisms for the active degradation of insulin[1,2] but the direct tissue analyses performed in the present studies, and by others,[28] would suggest that intraplacental sequestration of insulin may also occur. In both respects, placental mechanisms for insulin disposition seem to simulate those which have been described for the liver.[31–36] Whether the analogy may be carried further with regard to insulin effects upon glucose secretion remains to be elucidated. Nonetheless, from the available data, it is apparent that Claude Bernard's postulate of the placenta as the fetal liver should be extended to include its role in insulin metabolism.

*That TCA precipitation results in gross overestimation of intact insulin in the presence of plasma proteins has been amply documented.[3,5] Thus, although negative findings are significant, it is difficult to interpret other studies which have been presented in abstract form[29] in which TCA-precipitable radioactivity could be recovered in fetal blood following the administration of I^{131}-insulin to twenty-one-day pregnant rats.

SUMMARY

I^{131}-insulin has been employed to assess the permeability of the placenta of the twenty-one-day pregnant rat to insulin. Labeled hormone was administered into the maternal circulation by single injection or by prolonged infusion at constant rate. At various intervals following single injection or the establishment of distribution equilibrium by infusion, radioactive moieties in maternal plasma, fetal plasma, and in acid-alcohol extracts of fetus, placenta and maternal liver were characterized by chromatographic, electrophoretic or immunologic technics. Appreciable transplacental passage of insulin could not be demonstrated and concentrations of labeled hormone in the fetal circulation were less than 0.5 per cent of maternal values even after three hours of infusion. Contrariwise, significant quantities of intact I^{131}-insulin were present in placental extracts at all times and temporal relationships suggestive of intraplacental accumulation of hormone were observed. The data indicate that the placenta of the twenty-one-day pregnant rat represents a substantial barrier to the flux of insulin and may constitute the major target-site within the conceptus for maternal insulin.

SUMMARIO IN INTERLINGUA

Metabolismo de Hydrato de Carbon in Pregnantia: IV. Studios in Re le Permeabilitate del Placenta del Ratto pro Insulina Marcate con Iodo Radio-Active

Insulina a I^{131} esseva usate pro evalutar le permeabilitate del placenta pro insulina in rattas pregnante depost vinti-un dies. Le hormon in forma marcate esseva administrate ad in le circulation materne per un sol injection o per infusion prolongate a intensitate constante. A varie intervallos de tempore post injectiones solitari o post le establimento de equilibrio de distribution (in le caso de infusion), le componentes radioactive esseva determinate in le plasma materne, le plasma del feto, e in extractos a acido e alcohol ex feto, placenta, e hepate materne per medio de technicas chromatographic, electrophoretic o immunologic. Un appreciabile passage transplacental de insulina non poteva esser demonstrate, e le concentration del marcate hormon in le circulation del feto esseva minus que 0,5 pro cento del valor materne mesmo al fin de tres horas de infusion. Del altere latere, significative quantitates de intacte insulina a I^{131} esseva presente in extractos placental a omne tempores, e relationes chronometric esseva observate que pareva suggerer un accumulation intraplacental del hormon. Le datos indica que le placenta de rattas a pregnantia de vinti-un dies representa un forte barriera in le fluxo de insulina e constitue possibilemente le major sito de convergentia

pro insulina materne intra le complexo post-conceptional.

ACKNOWLEDGMENT

This investigation was supported in part by Research Grant A-1571 and Training Grant 2A-5060, National Institute for Arthritis and Metabolic Diseases, United States Public Health Service, Bethesda, Maryland, and the Howard Hughes Medical Institute. The authors are grateful to Dr. J. B. Josimovich and Dr. E. Knobil for the opportunity of seeing their manuscript on transplacental insulin exchange in the monkey in advance of publication.

REFERENCES

[1] Freinkel, N., and Goodner, C. J.: Carbohydrate metabolism in pregnancy. I. The metabolism of insulin by human placental tissue. J. Clin. Investigation *39*:116, 1960.

[2] Goodner, C. J., and Freinkel, N.: Carbohydrate metabolism in pregnancy: The degradation of insulin by extracts of maternal and fetal structures in the pregnant rat. Endocrinology *65*:957, 1959.

[3] Goodner, C. J., and Freinkel, N.: Carbohydrate metabolism in pregnancy: The turnover of I^{131}-insulin in the pregnant rat. Endocrinology *67*:862, 1960.

[4] Steele, R., Wall, J. S., DeBodo, R. C., and Altszuler, N.: Measurement of size and turnover rate of body glucose pool by the isotope dilution method. Am. J. Physiol. *187*:15, 1956.

[5] Berson, S. A., Yalow, R. S., Bauman, A., Rothschild, M. A., and Newerly, K.: Insulin-I^{131} metabolism in human subjects: Demonstration of insulin binding globulin in the circulation of insulin treated subjects. J. Clin. Investigation *35*:170, 1956.

[6] Narahara, H. T., and Williams, R. H.: Effect of protein added in vitro upon insulin degradation and glucose uptake by muscle. J. Biol. Chem. *233*:1034, 1958.

[7] Piazza, E. U., Goodner, C. J., and Freinkel, N. A.: A re-evaluation of in vitro methods for insulin bioassay. Diabetes *8*:459, 1959.

[8] Huggett, A. St. G., and Nixon, D. A.: Use of glucose oxidase, peroxidase, and o-dianisidine in determination of blood and urinary glucose. Lancet *2*:368, 1957.

[9] Somogyi, M.: Determination of blood sugar. J. Biol. Chem. *160*:69, 1945.

[10] Schwartz, I. L.: Measurement of extracellular fluid by means of a constant infusion technique without collection of urine. Am. J. Physiol. *160*:526, 1950.

[11] Pack, G., and Barber, D.: The placental transmission of insulin from fetus to mother. Am. J. Obst. & Gyn. *16*:115.

[12] Olow, J.: Über den Übergang von Insulin aus dem Fötus in die Mutter. Biochem. Zeitschr. *217*:475, 1930.

[13] Rupp, H.: Die Durchlässigkeit der Placenta und Eihäute für Antigene, Antikörper und Inkrete. Arch. f. Gynäkol. *143*: 80, 1930.

[14] Schlossman, H.: Beiträge zur Biologie der Plazenta. II. Mitteilung: Ist die Plazenta für Insulin durchlässig? Arch. f. exptl. Pathol. Pharmakol. Naunyn-Schmiedeberg's *159*:213.

[15] Corey, E. L.: Placental permeability to insulin in the albino rat. Physiol. Zoöl. *5*:36, 1932.

[16] Passmore, R., and Schlossman, H.: The effect of large doses of insulin on the foetal sheep and goat. J. Physiol. *92*: 459, 1938.

[17] Spoto, P.: Diabete e gravidanza. Passagio transplacentare dell' insulina. Minerva Ginec. *6*:61, 1954.

[18] Knobil, E., and Josimovich, J. B.: Placental transfer of thyrotropic hormone, thyroxine, triiodothyronine, and insulin in the rat. Ann. New York Acad. Sc. *75*:895, 1959.

[19] Weber, G., and Cantero, A.: Glucose-6-phosphatase activity in regenerating, embryonic and newborn rat liver. Cancer Research *15*:679, 1955.

[20] Kimmelstiel, R., and Villee, C. A.: Metabolism of carbon^{14}-labeled pyruvate by the newborn rat. Am. J. Physiol. *184*:63.

[21] Madison, L. L., Combes, B., Adams, R., and Strickland, W.: The physiological significance of the secretion of endogenous insulin into the portal circulation. III. Evidence for a direct immediate effect of insulin on the balance of glucose across the liver. J. Clin. Investigation *39*:507, 1960.

[22] Villee, C. A.: The metabolism of human placenta in vitro. J. Biol. Chem. *205*:113, 1953.

[23] Naeslund, J.: Investigations into the transit of reducing substances from mother to foetus in alimentary hyperglycaemia. Acta Obst. et Gyn. Scandinav. *7*:25, 1928.

[24] Runge, H., and Hartmann, H.: Untersuchungen über die Durchlässigkeit der Menschlichen Plazenta für Glukose. Zeitschr. f. Geburts. u. Gynäk. *96*:324, 1929.

[25] Villee, C. A.: Regulation of blood glucose in the human fetus. J. Appl. Physiol. *5*:437, 1953.

[26] Chinard, F. P., Danesino, V., Hartmann, W. L., Huggett, A. St. G., Paul, W., and Reynolds, S. R. M.: The transmission of hexoses across the placenta in the human and the rhesus monkey (macaca mulatta). J. Physiol. *132*:289, 1956.

[27] Hagerman, D. D., and Wellington, F. M.: Maternal and fetal liver metabolism in diabetic pregnant rats. Federation Proc. *17*:236, 1958.

[28] Roberts, W. J., Buse, M. G., and Buse, J.: The role of the human placenta in the transfer and metabolism of insulin. Clin. Res. *9*:30, 1961.

[29] Hwang, U. K., and Heggestad, C. B.: A study of the permeability of the rat placenta to radioactive I^{131}-insulin. Anat. Record *133*:290, 1959.

[30] Josimovich, J. B., and Knobil, E.: The placental transfer of I^{131}-insulin in the rhesus monkey. Am. J. Physiol. *200*:471, 1961.

[31] Mirsky, I. A., and Broh-Kahn, R. H.: The inactivation of insulin by tissue extracts. I. The distribution and properties of insulin inactivating extracts (insulinase). Arch. Biochem. *20*:1.

[32] Mirsky, I. A., Perisutti, G., and Dixon, F. J.: Destruction of I^{131}-labeled insulin by liver slices. Proc. Soc. Exp. Biol. (N.Y.) *86*:228, 1954.

[33] Tomizawa, H. H., Nutley, M. L., Narahara, H. T., and Williams, R. H.: Inactivation of insulin by rat liver extracts. J. Am. Chem. Soc. *76*:3357, 1954.

[34] Lee, N. D., and Williams, R. H.: The intracellular localization of labeled thyroxine and labeled insulin in mammalian liver. Endocrinology *54*:5, 1954.

[35] Madison, L. L., Combes, B., Unger, R. H., and Kaplan, N.: The relationship between the mechanism of action of the sulfonylureas and the secretion of insulin into the portal circulation. Ann. New York Acad. Sc. *74*:548, 1959.

[36] Mortimore, G. E., Tietze, F., and Stetten, D., Jr.: Metabolism of insulin-I^{131}. Studies in isolated, perfused rat liver and hind-limb preparations. Diabetes *8*:307, 1959.

Blood Viscosity, Serum Hexosamine and Diabetic Retinopathy

David G. Cogan, M.D., Lorenzo Merola, B.S., and Peter R. Laibson, M.D., Boston

The occurrence of microaneurysms and distention of veins, so characteristic of diabetic retinopathy, has suggested to many the likelihood of venous stasis as a cause of the angiopathy of diabetes. Yet direct measurement of the blood viscosity in diabetic patients with and without retinopathy has not, to our knowledge, been previously reported.* Several blood constituents, possibly bearing on viscosity, have, however, been studied in relation to retinopathy. These constituents include serum proteins,[1,2,3,4a,4b] lipoproteins,[5,6,2] cholesterol,[2] and polysaccharides.[7,3,8] The present study was undertaken to determine whether the measurable blood viscosity was higher in diabetics than in normals and whether there was a difference in diabetics with and without retinopathy; serum hexosamines were also determined on the same blood samples with the aim of correlating these with the blood viscosity.

METHODS

Viscosity

Determination of viscosity was made on blood taken from the arm vein of diabetic patients and normal donors. The blood was allowed to clot in a cold room at 5° C. and the serum separated by centrifugation. After removal of the serum, it was placed in a test tube in a constant temperature water bath at 25° C. for fifteen minutes.

The viscosity measurements were made by an Ostwald Fenske capillary type viscosimeter calibrated for 1 cc. volume. All measurements were made in a constant temperature bath at 25° C. One cubic centimeter of the blood serum which was equilibrated at 25° C. was placed in the viscosimeter and after another five-minute wait (to make sure the serum was at 25° C.), the serum was sucked up into the bulb of the capillary, and the flow between two marks (above and below the bulb) was timed. The procedure was repeated three times on the same serum sample. The average of the three determinations was taken as the rate of flow for the sample. Water was similarly equilibrated at 25° C. and its rate of flow determined in the same manner.

The viscosity of the serum relative to water was determined from the following formula:

$$\frac{\eta_{l2}}{\eta_{l1}} = \eta_{l}\text{rel}$$

where η_{l2} = rate of flow of sample; η_{l1} = rate of flow of water.

Hexosamine

Total hexosamine was determined by the Elson-Morgan method, as modified by Rimington.[9] Further slight modifications were made in this laboratory. To the precipitated serum protein, 2 ml. of 1N HCl was added. The tubes were sealed in vacuo, and the protein was hydrolyzed for eighteen hours at 110° C. The hydrolyzate was then neutralized with 2.25 N Na_2Co_3 until barely alkaline to litmus.

Optical densities were linear with varying amounts of standard hexosamine solution. In order to check the procedure, known quantities of hexosamine were added to the serum protein before hydrolysis. The hydrolysis with standard amounts of added hexosamine was then carried out under the procedure described.[10] In each case a recovery of 95-98 per cent hexosamine added was obtained. Determination of hexosamine was then carried out on each sample of blood.

RESULTS

The viscosity and hexosamine determinations are summarized in the accompanying table. The nine normal subjects were healthy persons of approximately the same age as the diabetics. The diabetics were, for the most part, patients under treatment at the Diabetes Clinic of the Massachusetts General Hospital and were thought to be under adequate control.

*Five diabetic patients with retinopathy were included in a series of blood viscosity measurements in patients with cerebrovascular disease (Swank, R. L.: Neurology 9:553, 1959). It was stated that these patients had an elevated blood viscosity but no comparison was made with diabetics who did not have retinopathy.

From the Howe Laboratory of Ophthalmology, Harvard Medical School, Massachusetts Eye and Ear Infirmary, 243 Charles Street, Boston 14, Mass.

The average blood viscosity was elevated in all of the diabetics (table 1). It was, however, not greater in diabetics with retinopathy than in those without retinopathy. The hexosamine levels were also elevated in the diabetics as compared to the controls but again not significantly more in those with retinopathy than those without retinopathy. Both viscosity and hexosamine measurements showed greater range of variation in the diabetics than in the normals.

TABLE 1

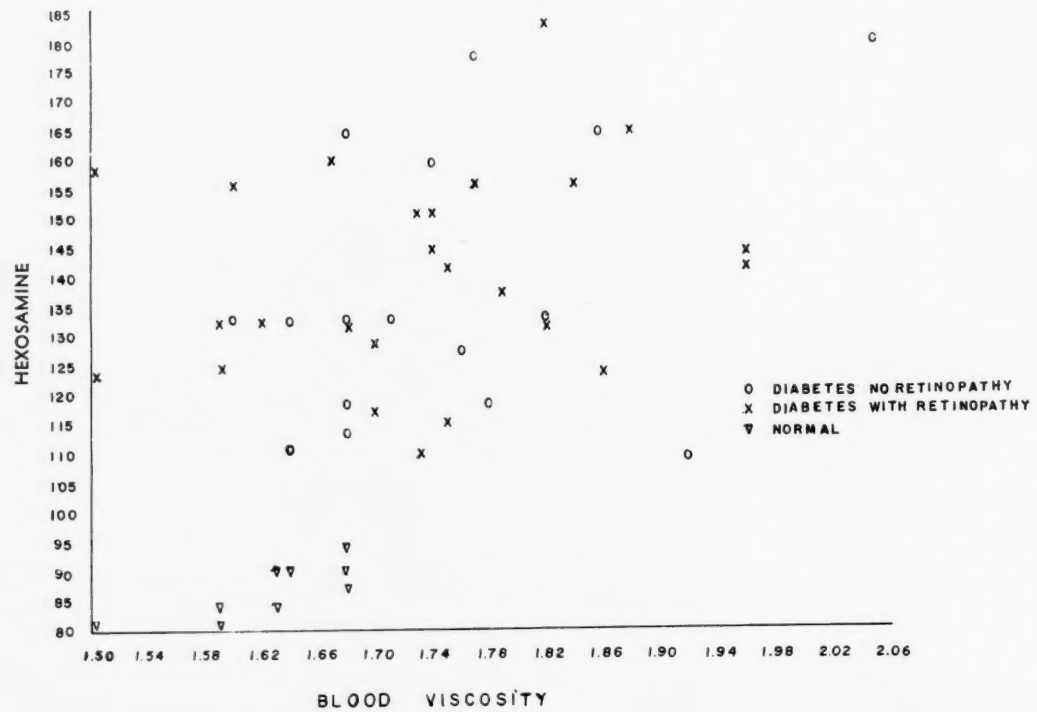
	Number of cases	Relative units viscosity	Mg. per cent hexosamine
Normal persons	9	1.62±0.02	86.6±4.5
Diabetics with no retinopathy	16	1.75±0.11	137.3±22.7
Diabetics with retinopathy	25	1.73±0.12	139.9±17.1

A scattergram (figure 1) shows a suggestive correlation of viscosity and hexosamine levels in the normal but a poor correlation in the diabetic.

COMMENTS

The possibility that the retinopathy of diabetes might be related to increased viscosity of the blood is not supported by the results of this study. Such a relationship, however, cannot be categorically denied, since others[11] have shown great fluctuations in diabetics (at least in coma) and single measurements obtained for the present study do not necessarily represent the range of viscosity changes. It is also remotely possible that the viscosity of the serum in the capillaries differs from that in the venous blood but this possibility seems unlikely. Also, it is possible that the elevated viscosity contributes to the pathogenesis of retinopathy but requires many years to have an appreciable effect. Such a possibility was not evident in the present series, for the alleged duration of the diabetes was not significantly different in those with and without retinopathy. What can be said from the present series is that long-standing diabetics with retinopathy do not have a maintained alteration in viscosity of their venous blood that differs significantly from diabetics without retinopathy.

Our findings of an elevation of hexosamines in dia-



betics agrees with that of some,[1,2,3,4a,4b] but not all,[13] others who have found elevated hexosamines in diabetics. The observation further agrees with that of others[3,4a,4b] who found no positive correlation with retinopathy.

The failure of the scattergram to show significant correlation of hexosamine and viscosity suggests that it is not the polysaccharides which are responsible for the elevated viscosity in diabetes. Other evidence might indict the serum lipids and lipoproteins.[14]

The failure to find elevated serum viscosity in diabetics with retinopathy as compared to that in diabetics without retinopathy is another negative correlation in this form of angiopathy. That elevated blood lipids or hexosamines could in themselves be responsible for the retinopathy seems unlikely in view of the absence of retinopathy in patients with marked and prolonged elevation of these constituents in nondiabetic conditions (idiopathic hyperlipemia, cancer, collagen diseases, et cetera).

SUMMARY

In twenty-five diabetics with retinopathy, sixteen diabetics without retinopathy, and nine normal controls, no significant correlation was found between alteration in blood viscosity and the presence of retinopathy. The hexosamine level in the blood was considerably elevated in the diabetics but no more so in those with retinopathy than in those without retinopathy. No correlation was found between blood hexosamine level and blood viscosity in diabetics.

SUMMARIO IN INTERLINGUA

Viscositate del Sanguine, Hexosamina del Sero, e Retinopathia Diabetic

In un studio de vinti-cinque diabeticos con retinopathia, de dece-sex diabeticos sin retinopathia, e de novem subjectos de controlo qui esseva normal, nulle significative correlation esseva constatate inter le viscositate del sanguine e le presentia de retinopathia. Le nivello sanguinee de hexosamina esseva considerabilemente elevate in le diabeticos, sed iste alteration non esseva plus marcate in le diabeticos con retinopathia que in le diabeticos sin retinopathia. Nulle correlation esseva trovate in le diabeticos inter le nivellos sanguinee de hexosamina e le viscositate del sanguine.

ACKNOWLEDGMENT

This study was supported by gift for Studies Pertinent to the Ocular Effects of Diabetes.

REFERENCES

[1] Schneider, R., Lewis, L. A., and McCullagh, E. P.: Plasma proteins: Alteration in diabetic retinitis. Am. J. Med. Sci. *212*:462, 1946.

[2] Hardin, R. C., Jackson, R. L., Cook, J. E., Johnston, T. L., Routh, J. I., and Kelly, H. G.: Alterations in serum globulins, cholesterol, and lipoproteins as related to diabetic retinitis. J. Lab. & Clin. Med. *42*:812, 1953.

[3] Keiding, N. R.: Levels of serum protein factors in diabetic patients with retinitis proliferans. Proc. Soc. Exper. Biol. & Med. *86*:390, 1954.

[4a] Lerman, S., Pogell, B. M., and Lieb, W.: Serum proteins and total glucosamine in diabetic retinopathy and glomerulosclerosis. Arch. Ophth. *57*:354, 1957.

[4b] Jahnke, K.: Diabetes Mellitus. Chapter in: Oberdisse, K., and Jahnke, K. (eds.) III Kongress der International Diabetes Federation Düsseldorf, 21. - 25. Juli, 1958. Proceedings —Verhandlungen—Comtes Rendus. Stuttgart, G. Thieme, 1959.

[5] Keiding, N. R., Mann, G. V., Root, H. F., Lawry, E. Y., and Marble, A.: Serum lipoproteins and cholesterol levels in normal subjects and in young patients with diabetes in relation to vascular complications. Diabetes *1*:434, 1952.

[6] Engelberg, H., Gofman, J. W., and Jones, H.: Serum lipids and lipoproteins in diabetic glomerulosclerosis: Preliminary observations of effect of heparin upon disease. Diabetes *1*:425, 1952.

[7] Berkman, J., Rifkin, H., and Ross, G.: Serum polysaccharides in diabetic patients with and without degenerative vascular disease. J. Clin. Investigation *32*:415, 1953.

[8] Maumenee, A. E., and Pogell, B. M.: Personal communication, 1958.

[9] Rimington, C.: Seromucoid and the bound carbohydrate of the serum proteins. Biochem. J. *34*:931, 1940.

[10] Glick, D.: Methods of biochemical analysis, Vol. 2. New York, Interscience Publishers, 1955, pp. 293-95.

[11] Reubi, F. C.: Glomerular filtration rate, renal blood flow and blood viscosity during and after diabetic coma. Circulation Research *1*:410, 1953.

[12] Jacobs, H. R.: Bound glucosamine of serum mucoid in diabetes mellitus: Fluctuations observed under influence of insulin. J. Lab. & Clin. Med. *34*:116, 1949.

[13] West R., and Clarke, O. H.: Concentration of glucosamine in normal and pathological sera. J. Clin. Investigation *17*:173, 1938.

[14] Swank, R. L.: Effects of fat on blood viscosity in dogs. Circulation Research *4*:579, 1956.

EDITORIAL

NEWER CONCEPTS OF ATHEROSCLEROSIS

Atherosclerosis, by far the most important form of arteriosclerosis, is characterized by the formation of fatty and fibrous plaques in the intima of the larger arteries. It is the number one killer among nondiabetics as well as diabetics, but the incidence of aberrations of carbohydrate metabolism among persons with severe atherosclerosis is extraordinarily high.[1]

Research on this most important disease of civilized man has, in recent years, been devoted so overwhelmingly to the study of the blood lipids that one might almost say that a formula had been evolved: too much of some kind of lipid in the diet, hence too much in the blood, hence too much in the vessel wall. That such simplification is probably far from the truth is emphasized in a recent lucid and balanced review.[2]

Recently there seems to have been a welcome reawakening of interest in the anatomy and physiology of the arterial wall[3] and in some of the nonlipid constituents of the atheromatous plaque. In relation to the latter, two lines of thought which have been given scant attention in this country have appeared lately in the foreign literature.

The first of these, which has been set forth chiefly by Duguid,[4,5] has come to be called the thrombogenic theory and holds that mural thrombi may become incorporated into the lining of the artery in such a way that eventually a typical fibrous or fatty atheromatous plaque results. It does not necessarily follow that all plaques arise in this way, but evidence is increasing that many do. The theory is being broadened by the demonstration of small quantities of fibrin in very small, and presumably early lesions by the fluorescent antibody technic[6] and other methods,[7] and by application of dynamic concepts of a balance between the deposition of fibrin and fibrinolysis.[8] Also, it is being recognized that such deposits of fibrin may be related to, or result from, extravasations of blood in the intima.[9] The lack of attention given to the thrombogenic theory in American textbooks and symposia on atherosclerosis (with the outstanding exception of the Princeton conference[8]) is all the more surprising when one realizes that many of Duguid's conclusions were anticipated, not only by Rokitansky,[10] but also by the careful observations of Clark, Graef and Chasis[11] and of Horn and Finkelstein.[12]

The second line of investigation, which, so far as the present writer is aware, has not even been mentioned in the American literature, is that of Randerath and Diezel.[13,14] These authors, using special dyes and polarized light, and studying the muscular arteries rather than the aorta, claim to have demonstrated fibrillar, highly polymerized mucopolysaccharides in greater abundance in diabetics than in nondiabetics. Such lesions are so characteristic that they have dubbed them "MPS plaques," and have postulated that, as a result of insulin deficiency, protein-bound hexoses and hexosamines of the blood accumulate in the intima of the artery and eventually become transformed from slightly polymerized to highly polymerized compounds. Thus the metabolic disturbance in diabetes is tied directly to the atheromatous plaque (perhaps successfully, for the first time). It is, of course, possible that these deposits may be secondary, as suggested by some experimental work[15] and even that they may tend, by their anticoagulant properties, to prevent further thrombi in such loci.[16] However, if confirmed, the work of Randerath and Diezel should be of special value in contributing toward a unifying concept of the pathogenesis of the so-called complications of diabetes.

It is possible that neither of these two approaches to the study of atherosclerosis will prove, in the years ahead, to have great validity or significance. On the other hand, they may provide, temporarily, refreshing relief from the current preoccupation with cholesterol and lipoproteins.

REFERENCES

[1] Waddell, W. R., and Field, R. A.: Carbohydrate metabolism in atherosclerosis. Metabolism 9:800, 1960.

[2] Hilditch, T. P., and Jasperson, H.: Lipids in Relation to Arterial Disease, Distributed by J. Bibby and Sons, Ltd., 1959. Liverpool, England.

[3] Lansing, A. I. (ed.): The Arterial Wall. Baltimore, Williams and Wilkins, 1959.

[4] Duguid, J. B.: Thrombosis as a factor in the pathogenesis of coronary atherosclerosis. J. Path. & Bact. 58:207, 1946.

[5] Duguid, J. B.: Chapter 22 in Cowdry's Arteriosclerosis, ed. by H. T. Blumenthal. 1961 (in preparation).

[6] Woolf, N., and Crawford, T.: Fatty streaks in the aortic intima studied by an immuno-histochemical technique. J. Path. & Bact. 80:405, 1960.

[7] More, R. H., and Haust, M. D.: Atherogenesis and plasma constituents. Am. J. Path. 38:527, 1961.

[8] Astrup, T.: Rôle of blood coagulation and fibrinolysis in the pathogenesis of arteriosclerosis. In Conference on Connective Tissue, Thrombosis and Atherosclerosis, held in Princeton, N. J. Ed. by I. H. Page. New York, Academic Press, 1959.

[9] Leading article. Intimal haemorrhage. Brit. M. J. 1:961, 1959.

[10] Rokitansky, C.: A Manual of Pathological Anatomy. Translated from the German by C. W. Moore. Philadelphia, Blanchard-Lea, 1855, vol. 4, p. 200.

[11] Clark, E., Graef, I., and Chasis, H.: Thrombosis of the aorta and coronary arteries, with special reference to the "fibrinoid" lesions. Arch. Path. 22:183, 1936.

[12] Horn, H., and Finkelstein, L. E.: Arteriosclerosis of the coronary arteries and the mechanism of their occlusion. Am. Heart J. 19:655, 1940.

[13] Randerath, E., and Diezel, P. B.: Morphologische Pathologie der extrarenalen Angiopathie beim Diabetes mellitus. Proceedings of the Third Congress of the International Diabetes Federation, Dusseldorf, 1958. Stuttgart, Geo. Thieme, 1959, p. 54.

[14] Randerath, E., and Diezel, P. B.: Vergleichende histochemische Untersuchungen der Arteriosklerose bei Diabetes mellitus und ohne Diabetes mellitus. Deutsches Arch. f. klin. Med. 205:523, 1959.

[15] Bollet, A. J., Wang, C., and Adlersberg, D.: Experimental atheromatosis: acid mucopolysaccharide content of the aorta. Circulation Res. 8:88, 1960.

[16] Gore, I., and Larkey, B. J.: Functional activity of aortic mucopolysaccharides. J. Lab. & Clin. Med. 56:839, 1960.

PHILIP M. LE COMPTE, M.D.
Boston

BOOK REVIEWS

DIABETES MELLITUS: A HANDBOOK FOR NURSES. *By Marguerite M. Martin, R.N. $3.50, pp. 167, W. B. Saunders, Co., Philadelphia and London, 1960.*

The author has written a handbook for nurses which touches upon all phases of diabetes. In this endeavor she has had a unique advantage and stimulus in that her husband has had diabetes for over thirty years. She has carefully and comprehensively outlined the opportunities, duties and problems confronting nurses in the management of diabetes.

Nurses should know the symptoms of diabetes so well that they are able to recognize the condition quickly. It is necessary for them to know all new drugs in general use. The nurse should understand that because a diabetic individual falls into a certain age group, or patient type this does not necessarily indicate that he will respond to treatment with the oral agent usually applicable to his group. Moreover, nurses need to be familiar with possible side effects of the oral hypoglycemic drugs as well as insulin, so that they will be able to call them promptly to the physician's attention. Very close surveillance of postoperative diabetic patients by the nurse is necessary.

In accordance with modern broad concepts, nurses must recognize their responsibility to the community in teaching prevention, and detection. They can assist in the detection drives conducted by the American Diabetes Association each November. Industrial nurses in particular have an opportunity to play a part of inestimable value in the detection of unknown diabetics. Because of the confidence and affection engendered in employees over a period of years, the industrial nurse is able to assist, teach, and encourage diabetic workers who are concerned or confused about their condition or its management.

As pointed out in this publication a great deal of education in diabetes should be assigned to nurses in their various capacities. Although there are numerous handbooks for diabetic patients written in simplified language, this book is very suitable for background material for the nurse who is concerned with education since it is the product of a nurse experienced in this area.

AN OUTLINE OF CHEMICAL GENETICS. *By Bernard S. Strauss, Ph.D. $5.00, pp. 188, W. B. Saunders, Philadelphia & London, 1960.*

This excellent book summarizes the chief developments in the rapidly advancing field of chemical genetics. Dr. Strauss cites the evidence for deoxyribonucleic acid (DNA) being the code-carrying material that is transmitted in heredity. He gives a concise picture of the helical structure and chemical constitution of the DNA molecule, with evidence in support of the Watson-Crick model and its proposed mechanism of replication. There is a good description of what may constitute a gene from the operational and chemical standpoint, and how it may carry the hereditary code and transmit it. Also well described are the template relationships between DNA and RNA (ribonucleic acid) and the proposed mechanisms by which the arrangement of purine and pyrimidine bases in these structures may order the sequence of amino acids that regulate and determine which protein is synthesized. An entire chapter is devoted to current concepts of the mechanism of protein and hence enzyme synthesis. Mutations and their possible mechanisms of production at the chemical or gene level are described.

There is a short chapter only on the biochemical genetics of man; but there is a concise review of three well-chosen examples of inborn errors of metabolism or human genetic disease about which most is known concerning the precise enzymatic defects or alteration in protein structure, which illustrates well the concept of molecular disease. This gives insight into the manner by which a single gene—enzyme or gene—polypeptide defect can produce a multiplicity of apparently unrelated symptoms and signs (pleiotrophy) in a specific disease.

The subject matter covered here lays a groundwork for understanding much of the recent progress in human genetics and medical genetics, accomplishments that, coupled with those that have been achieved in human cytogenetics, are providing a fresh approach to the causation and pathogenesis of many diseases. In other instances they give us new insight into the mechanisms underlying many of the variable responses, both beneficial and toxic, that occur with drug therapy.

ABSTRACTS

Angervall, Lennart; Dotevall, Gerhard; and Lehmann, Klas-Erik (Dept. of Path. II and Med. Dept. I and II, University of Göteborg, Sahlgren's Hosp., Göteborg, Sweden): THE GASTRIC MUCOSA IN DIABETES MELLITUS: A FUNCTIONAL AND HISTOPATHOLOGICAL STUDY. Acta med. scandinav. *169*: 339-49, March 1961.

In fifty-six diabetics the secretion of hydrochloric acid was studied after maximum stimulation with histamine. In eighteen cases achlorhydria or hypochlorhydria was present. Ten of them were available for further studies, reported in the present paper. The investigation comprised Schilling tests, determinations of the uropepsin excretion and the serum levels of vitamin B_{12}, and hematologic examinations. In eight cases peroral gastric biopsies were performed. Chronic atrophic gastritis was histologically demonstrated in all cases with achlorhydria or hypochlorhydria and was associated with blood vessel lesions in the tunica mucosae. The demonstrated lesions were caliber reduction, thickened walls and PAS-stainability. The authors' opinion is that nutritional disturbances in the gastric mucosa caused by the vessel lesions may be a contributing factor in the development of chronic atrophic gastritis in diabetics. Other possible factors such as nervous and hormonal disturbances and states of deficiency of iron or vitamins are also discussed. In three cases with atrophic gastritis latent or manifest pernicious anemia was demonstrated. It is suggested that an increased frequency of chronic atrophic gastritis is responsible for the probably high incidence of achlorhydria and pernicious anemia, reported in diabetics. B.F.K.

Armin, J.; Cunningham, N. F.; Grant, R. T.; Lloyd, M. K.; and Wright, P. H. (Dept. of Chem. Path. and Exper. Med., Guy's Hosp. Med. Sch., London, S.E. 1; and the Ministry of Agriculture, Fisheries and Food, Central Veterinary Lab., New Haw, Weybridge, Surrey, England): ACUTE INSULIN DEFICIENCY PROVOKED IN THE DOG, PIG AND SHEEP BY SINGLE INJECTIONS OF ANTI-INSULIN SERUM. J. Physiol. *157*:64-73, June 1961.

Antibodies to crystalline bovine insulin were produced in guinea pigs. Intravenous injection of anti-insulin serum into two dogs, a pig and a lamb produced hyperglycemia lasting for several hours. A third dog did not exhibit hyperglycemia. In the case of the lamb, bioassays using the rat hemidiaphragm glucose uptake method revealed a marked decrease in insulin-like activity of the circulating blood following injection of anti-insulin serum. H.T.N.

Beaser, Samuel B. (Harvard Med. Sch. and Med. Serv. of Beth Israel Hosp., Boston, Mass.): FURTHER STUDIES ON THE ACTION OF CHLORPROPAMIDE IN DIABETES MELLITUS. Clin. Pharmacol. & Therap. *2*:157-62, March-April 1961.

This is a continuation of previous studies on the relationship between dosage, blood levels, and clinical action of chlorpropamide. W.R.K.

Beaser, Samuel B. (Harvard Med. Sch. and Beth Israel Hosp., Boston, Mass.): ORALLY GIVEN COMBINATIONS OF DRUGS IN DIABETES MELLITUS THERAPY: FURTHER TRIAL. J.A.M.A. *174*:2137-41, Dec. 24, 1960.

A total of twenty-five ambulatory diabetic patients were treated with a combination of phenformin and a sulfonylurea drug (either tolbutamide or chlorpropamide). These patients had failed to respond fully to treatment with either sulfonylurea drug alone. The therapy was successful in reducing blood sugar to satisfactory levels in nineteen patients, but three had to discontinue the treatment because of gastrointestinal intolerance. The therapy was unsuccessful in six patients, largely because of severity of the diabetes. B.F.K.

Bhattacharya, G. (Dept. Biochem., University of Cambridge, England): EFFECTS OF METAL IONS ON THE UTILIZATION OF GLUCOSE AND ON THE INFLUENCE OF INSULIN ON IT BY THE ISOLATED RAT DIAPHRAGM. Biochem. J. *79*:369-77, May 1961.

The effect of insulin in increasing glucose utilization by the isolated rat diaphragm is obtained only in the presence of certain metal ions. The simultaneous presence of Mg++ and Na+ is required for the maximum insulin effect. The effect of Mg is possibly due to preservation of the integrity of the cell surface and is antagonized by Ca.

The alkali-metal ions have a striking regulatory effect on the basal uptake of glucose by the diaphragm. Highest uptakes were recorded in the presence of Li and decreased in the following order: Li, Na, K, Rb and Cs. The regulatory effects of these ions persists even when the tissue has permanently lost its ability to respond to insulin, and may be observed by preincubation of tissues in various ionic solutions with subsequent incubation and determination of glucose uptake in Gey's solution.

Determination of the xylose space of tissues incubated in various solutions suggests that the alkali-metal ions may act to regulate the rate of entry of the sugar from the extracellular fluid to the interior of the cell. It is suggested that two separate processes are possibly involved in the utilization of glucose by the rat diaphragm: one insulin dependent, the other insulin independent. J.A.

Bloom, Walter Lyon; and Ward, John A. (Res. Lab., Piedmont Hosp., Atlanta, Ga.): CHANGES IN CARBOHYDRATE METABOLISM DURING BLOOD LOSS SHOCK AND ANOXIA. Metabolism *10*:379-85, May 1961.

The authors observed no significant changes in blood sugar in adrenalectomized or intact rats during progressive blood loss (0.5 per cent of body weight removed every twenty minutes) or anoxia (3 per cent oxygen, 97 per cent nitrogen). Hepatectomy resulted in gradual decline in blood sugar not seen in intact animals. This decline was accelerated by hemorrhage and anoxia. In the intact rat, blood lactic acid rose with blood loss. Anoxia had a more pronounced effect. In the dehepatized animal, the rise in lactic acid was intensified by both blood loss and anoxia, and was more pronounced than in

the anoxic intact animal. Glycogen changes during blood loss were most pronounced in the liver, in which there was a rapid decrease during blood loss but no change with anoxia. Cardiac and leg muscle glycogen changed little during blood loss in the fasted rat, whereas the acid-soluble glycogen increased in the fed rat. The changes in tissue glycogen, blood sugar and lactic acid in adrenalectomized rats were no different from those of intact rats subjected to blood loss. In view of the difference in hepatic glycogenolysis observed in blood loss and anoxia, the change in cellular physiology in anoxia is concluded to be different from that of ischemia despite similarities of blood changes in the two conditions. Anoxia is only one phase of the disturbance at the cellular level when circulation fails. The important role of alterations in hepatic metabolism in shock is re-emphasized. C.A.R.

Bolinger, Robert E.; Tu, Wu-Hao; and Kendall, Charles (Dept. of Med., Univ. of Kansas, Sch. of Med., Kansas City, Kans.): ORAL HYPOGLYCEMIC AGENTS: CLINICAL EXPERIENCE WITH 50 DIABETIC PATIENTS. J. Kansas M. Soc. 61:135-40, March 1960.

Experiences with management of fifty diabetic patients with oral hypoglycemic agents is reported. A good response was obtained in about half of the patients treated with the sulfonylureas and failure occurred in about one fifth of the cases tried. The incidence of successes increased after age forty-five and in patients taking less than 20 units of insulin. Several incidences of refractoriness to one of the sulfonylurea preparations and responsiveness to another were noted. DBI had the additional advantage over the sulfonylureas in being effective in combination with insulin in young, "brittle" diabetics. Reactions to be guarded against with the sulfonylurea drugs are hypoglycemia and ketosis in refractory patients. B.F.K.

Brandt, Lars; Nordén, Åke; and Swahn, Bengt (Dept. of Med., Univ. Hosp., Lund, Sweden): ASSESSMENT OF TREATMENT WITH TOLBUTAMIDE IN DIABETES MELLITUS. Acta med. scandinav. 169:193-200, February 1961.

Tolbutamide was instituted in sixty-six patients during the period Jan. 1, 1956, to Dec. 31, 1957. The patients were followed until March of 1960. Six patients died during the investigation. Carcinoma of the pancreas was revealed at autopsy in two patients and one alive is also known to have pancreatic carcinoma. In a group of fourteen patients treated for twenty-five to twenty-nine months, eight have required insulin treatment for adequate control. In another group of sixteen patients treated for thirty to forty-four monhs, insulin was necessary in eight. It appears that with time tolbutamide treatment represents adequate treatment for only a few patients. Close control is necessary and the effect on the patient's general state of health when tolbutamide is replaced by insulin frequently gives the best illustration of the ineffectiveness of oral treatment. B.F.K.

Craig, James W.; Drucker, William R.; Miller, Max; and Woodward, Hiram, Jr. (Dept. of Med. & Surg., Sch. of Med., Western Reserve Univ., Cleveland, Ohio): A PROMPT EFFECT OF EXOGENOUS INSULIN ON NET HEPATIC GLUCOSE OUTPUT IN MAN. Metabolism 10:212-20, March 1961.

The authors report the effect of insulin administration on net hepatic glucose output by utilizing hepatic vein catheterization in three patients with liver cirrhosis and end-to-side portacaval venous anastomoses (two of them were diabetic). Peripheral intravenous administration of single doses of 6 to 10 units of glucagon-free insulin was followed in five to ten minutes by a reduction in net hepatic glucose output. Two of the subjects demonstrated an average net hepatic glucose uptake during the fifty minutes after insulin administration. In these experiments, a prompt effect of insulin on directly measured net hepatic glucose output was demonstrated. C.A.R.

Daughaday, William H. (Dept. of Med., Washington Univ., Sch. of Med., St. Louis, Mo.): HYDROGEN ION METABOLISM IN DIABETIC ACIDOSIS: DISTURBANCES BEFORE AND AFTER TREATMENT. Arch. Int. Med. 107:63-68, January 1961.

The consequences of hydrogen ion overproduction as it occurs in uncontrolled diabetes have been analyzed. Although considerable information exists concerning the buffering activity of plasma and erythrocytes, much less is known concerning the hydrogen exchanges which occur in cells and bone. Impaired β-oxybutyric acid oxidation and impaired renal excretion of hydrogen ions may accelerate hydrogen ion accumulation in the body in diabetic acidosis. Inhibition of hepatic release of β-oxybutyric acids by restoration of normal hepatic cell carbohydrate metabolism is fundamental to clinical recovery. The acceleration of this recovery by the administration of additional carbohydrate has been demonstrated in controlled studies of human diabetic ketosis. Two types of iatrogenic alkalosis occur in recovery from diabetic acidosis, and both are related to delayed recovery of the cellular "internal" environment. Respiratory alkalosis occurring relatively early in the course of treatment may be due to persisting acidosis within the respiratory center. Later, a metabolic alkalosis may occur due to impaired bicarbonate excretion related to potassium deficiency in the renal tubular cells. B.F.K.

Daysog, Alberto, Jr.; Dobson, Harold L.; and Brennan, James C. (Dept. of Med. & Path., Baylor Univ. Coll. of Med. & Jefferson Davis Hosp., Houston, Tex.): RENAL GLOMERULAR AND VASCULAR LESIONS IN PREDIABETES AND IN DIABETES MELLITUS: A STUDY BASED ON RENAL BIOPSIES. Ann. Int. Med. 54:672-84, April 1961.

The authors studied the renal tissue of sixty-two patients with abnormalities of carbohydrate metabolism ranging from a deficiency in glucose tolerance test elicitable only after steroid stress to frank insulin treated diabetes of many years' duration. Percutaneous biopsy was the source of renal tissue and subject to various stains and electron microscopy.

All patients showed glomerular basement membrane thickening, 50 per cent the classical Kimmelstiel-Wilson nodular lesions, 61 per cent fibrin caps and capsular drops and 50 per cent pyelonephritis. The presence of the first lesions was confirmed by electron microscopy.

The severity of the renal lesions did not bear any constant relationship to that of the severity or duration of the metabolic defect. This was considered as evidence in favor of the concept that the two were independent of each other though evolving from "the same basic metabolic abnormality." S.B.B.

Desjardins, Jay A.; and Brod, Robert C. (Dept. of Int. Med., U. S. Naval Hosp., Philadelphia, Pa.): PHEOCHROMOCYTOMA AND DIABETES MELLITUS: REPORT OF A CASE WITH TRAN-

SIENT EXACERBATION OF DIABETIC RETINOPATHY FOLLOWING REMOVAL OF THE TUMOR. Ann. Int. Med. *54*:998-1008, May 1961.

A fifty-one-year-old patient with recent diabetes and a longer history of hypertension, weight loss and increased sweating was found to have a pheochromocytoma. Diabetic retinopathy of mild degree was noted, with microaneurysms and exudates.

After removal of the retroperitoneal pheochromocytoma, the hypertension and diabetes were immediately ameliorated, but the retinopathy worsened in the first two weeks after operation. However, six and then twelve weeks after operation there was marked improvement of all fundoscopic findings. S.B.B.

Donnell, George N.; Collado, Manuel; and Koch, Richard (Dept. of Pediat., Univ. of Southern California Sch. of Med., Los Angeles, Calif.): GROWTH AND DEVELOPMENT OF CHILDREN WITH GALACTOSEMIA. J. Pediat. *58*:836-44, June 1961.

A study of sixteen families with a total of twenty-four galactosemic children is reported, with longitudinal observations of mental and physical development in fifteen of the children. The data suggest that dietary restriction of galactose permits a child with galactosemia to achieve a pattern of growth comparing favorably in both height and weight with that of normal children. No firm conclusions could be drawn from the data regarding mental development. Four of the children are mentally defective and one is in a state hospital for mentally defective children. Six have borderline intelligence, six are in the low normal range, and only three have I.Q. ratings over one hundred. Late inauguration of dietary management was associated with a greater incidence and severity of mental handicap. R.L.J.

De Ropp, R. S.; and Snedeker, E. H. (Biochem. Res. Dept., Biochem. Res. Section, Lederle Labs., Pearl River, N. Y.): EFFECT OF DRUGS ON AMINO ACID LEVELS IN THE RAT BRAIN: HYPOGLYCEMIC AGENTS. J. Neurochem. 7:128-34, May 1961.

The effect of insulin on the amino acid levels of the brain has been attributed to changes in the amounts of citric acid cycle intermediates consequent to hypoglycemia. A lowering in the glucose level of the brain would result presumably in a lowering of pyruvate. Oxalacetic acid would tend to accumulate because, in the absence of sufficient pyruvate, it would not be condensed to citrate. Treatment of fasted rats with insulin brought about a characteristic change in the levels of certain ninhydrin-reacting substances in the brain: a rise in aspartic acid and ethanolamine, a fall in glutamic acid, glutamine, alanine, GABA (γ-aminobutyric acid), and glycine. Glutathione, phosphoethanolamine, and taurine were not affected. Oral hypoglycemic agents, tolbutamide, hypoglycin A, and the keto acid of hypoglycin A, did not produce any of the changes in amino acid levels characteristic of insulin, probably because they did not reduce blood sugar levels enough to produce coma. Their only significant effect was to raise the level of GABA. W.R.K.

DiGeorge, Angelo M.; Auerbach, Victor H.; and Mabry, C. Charlton (Dept. Pediat. and Physiol. Chem., Temple Univ. Sch. of Med., and St. Christopher's Hosp. for Children, Philadelphia, Pa.): ELEVATED SERUM INSULIN ASSOCIATED WITH LEUCINE-INDUCED HYPOGLYCEMIA. Nature *188*:1036-37, Dec. 17, 1960.

It would appear that in the majority of children sensitive to leucine the blood-sugar depressant activity of this amino-acid is mediated via increased synthesis and/or release of insulin. The exact mechanism by which leucine increases the circulating insulin level is unknown. It is remarkable that in one of our two patients, hypoglycemia followed the administration of leucine without an associated significant increase in insulin level.

The discrepancy may be explained by the presence of circulating insulin in a "bound" form not readily detectable by the technics employed. Other possible explanations also are now being tested. W.R.K.

Ditschuneit, H.; Ziegler, R.; and Pfeiffer, E. F. (I. Medical Dept. Univ. Frankfurt a.m., Germany): ON THE BLOOD INSULIN ASSAY. V. THE EFFECT OF BOVINE AND HUMAN GROWTH HORMONE AND OF OTHER HORMONES ON THE CARBOHYDRATE METABOLISM OF THE ISOLATED EPIDIDYMAL FAT PAD OF THE RAT. Klin. Wchnschr. *39*:426-30, April 15, 1961.

The authors investigated the effect of human and bovine growth hormone, glucagon, cortisone, 6 methyl-prednisolone, tri-iodothyronine and tri-iodothyroacetic acid on the carbohydrate metabolism of the isolated epididymal fat pad of the rat. The tissue was suspended in buffer solution and studies were carried out with and without the addition of 1,000 micro-units of crystalline insulin. In addition, experiments were carried out to investigate the influence of these various hormones on the serum insulin-like activity. It was found that none of the studied hormones except insulin influenced the oxidation of 1-C^{14}-glucose to $C^{14}O_2$. This oxidation was increased eightfold by 1,000 micro-units of crystalline insulin. Glucose entrance was increased by 380 per cent with the addition of insulin, while 0.1 γ/ml. glucagon caused an increase only of 52 per cent, 0.1 γ/ml. cortisone of 25 per cent, and 0.25 γ/ml. tri-iodothyronine of 75 per cent. Glucagon, cortisone and tri-iodothyronine augment to a small degree the effect of insulin on glucose entrance, but do not influence at all its effect on glucose oxidation. In contrast to bovine growth hormone which is ineffective, human growth hormone was found to have the most significant influence on the insulin effect. Human growth hormone (100 γ/ml.) augmented by 125 per cent the insulin effect on glucose entrance and by 31 per cent its effect on glucose oxidation. It is concluded that the oxidation of 1-C^{14} glucose to $C^{14}O_2$ in the isolated epididymal fat pad results exclusively from the effect of insulin and that this method is useful for the assay of the biologically active insulin in serum. (German) M.G.G.

Duncan, Garfield G.; Jenson, William; and Eberly, Richard J. (Div. of Med., Pennsylvania Hosp., Philadelphia, Pa.): FACTITIOUS HYPOGLYCEMIA DUE TO CHLORPROPAMIDE: REPORT OF A CASE, WITH CLINICAL SIMILARITY TO AN ISLET CELL TUMOR OF THE PANCREAS. J.A.M.A. *175*:904-06, March 11, 1961.

After careful investigation, a thirty-eight-year-old woman with the clinical characteristics of insulinoma was operated. There was no tumor or other abnormality found. A second exploratory operation was contemplated when a cache of chlorpropamide was found in her room.

A high chlorpropamide concentration was present in the patient's serum. B.F.K.

Editorial: RENEWED INTEREST IN ASPIRIN AS A HYPOGLYCEMIC AGENT. J.A.M.A. *175*:1173-74, April 1, 1961.

An editorial summarizing the renewed interest in aspirin as a hypoglycemic agent. B.F.K.

Egeli, E. S.; and Alp, H. (Med. Dept., Univ. Istanbul, Turkey): ON THE EFFECT OF CARBUTAMIDE ON THE CARBOHYDRATE METABOLISM. Klin. Wchnschr. *39*:437-40, April 15, 1961.

The authors examined the glucose-6-phosphatase activity in liver tissue of twenty diabetic and six nondiabetic patients, before and after carbutamide medication. Liver tissue was obtained by needle biopsy. They also determined the simultaneous blood sugar levels in peripheral blood (fingertip) and the vena hepatica (catheter blood) before and after the intravenous administration of 1.5 gm. carbutamide. In a few cases of this series, 1 mg. glucagon was given intravenously twenty to thirty minutes prior to the injection of the carbutamide. It was found that carbutamide decreases markedly both the glucose-6-phosphatase activity in liver tissue and the hepatic glucose release. These observations were made both in the diabetic and the nondiabetic patients. Carbutamide also inhibited the hepatic glucose mobilization of glucagon. The rapid action of carbutamide suggests a direct effect on the liver; it can, however, not be excluded that this compound acts via insulin release. (German) M.G.G.

Feder, Isidore A.; and Kahn, Alvin (Dept. of Med., Beth-El Hosp., Brooklyn, N. Y.): CLINICAL EFFECTIVENESS AND TOXICITY OF METAHEXAMIDE IN TREATMENT OF DIABETES MELLITUS. Metabolism *10*:246-60, March 1961.

The authors administered metahexamide to twenty-two patients (ages twenty-two to eighty-two) with diabetes, twenty-one of whom were stable diabetics. Good diabetes conrol was attained in only 50 per cent of the patients. Duration of treatment ranged from two weeks to five months. The usual dose of metahexamide was 100-200 mg. daily. Hepatic dysfunction was observed in ten patients, three of whom showed hepatic damage by liver biopsy. Jaundice occurred in one patient. Less serious side effects, such as weakness, anorexia, nausea, diarrhea, vertigo, pruritis, dermatitis and eosinophilia, developed in eight patients. The authors concluded that this drug is not completely effective as a hypoglycemic agent and is too toxic to be utilized in diabetes treatment. C.A.R.

Field, Richard A.; Hall, Wm. A.; Contreras, J. S.; and Sweet, Wm. H. (Harvard Med. Sch., Boston, Mass.): HYPOPHYSEAL-STALK SECTION IN THE TREATMENT OF ADVANCING DIABETIC RETINOPATHY—REPORT OF THREE CASES. New England J. Med. *264*:689, April 6, 1961.

This paper deals with three patients, with diabetic retinopathy of a severe degree with immediate threat to useful vision, who were subjected to hypophyseal-stalk section. The advance of retinal disease appears to have been arrested in that hemorrhagic activity has not been apparent since operation, and shrinkage of aneurysmal lesions, proliferated tissue and neovascularization has taken place. Postoperatively transient diabetes insipidus and evidence of anterior pituitary insufficiency developed in all cases but these complications were not serious. Criteria for the selection of the patients included documented progressive retinopathy, the tempo of advance of which threatened loss of useful vision within a relatively short predictable period. Cases of long-standing macular destruction, total retinal detachment or partial detachment with breaks in the retina were excluded. Also excluded were cases in which proliferative changes had advanced to the point of irreversible fibrosis. Cases of long-standing, relatively stationary retinopathy were excluded. Demonstrated inability to retard the advance of the retinal processes by the strictest medical management achievable with complete patient cooperation was also another important criterion. For acceptance, cases were required to have demonstrated absence of advanced or significant renal impairment due to diabetic nephropathy and demonstrated absence of incapacitating cardiac peripheral vascular or neurologic disease. Rapid clearing of blood from the vitreous suggests the presence of a humoral factor of hypothalamic or pituitary origin in the pathogenesis of diabetic retinopathy according to these authors. The beneficial results afforded these three patients warrant consideration of this procedure in selected patients and point to the necessity for continuing investigations into the neuro-endocrine pathophysiology of diabetic vascular disease. S.S.

Fineberg, S. K. (Med. Serv., Harlem Hosp., New York, N. Y.): OBESITY-DIABETES AND ANOREXIGENICS. J.A.M.A. *175*:680-84, Feb. 25, 1961.

The primary therapeutic target in the management of obesity-diabetes is, and always must be, the obesity. The use of oral hypoglycemic medicaments for this type of diabetes is a therapy-of-convenience which should be deplored. Such therapy should only be tolerated after long and conscientious application of all possible means to effect weight reduction. A carefully controlled, "triple-blind" crossover study comparing the anorexigenic and central nervous system effects of phenmetrazine hydrochloride (Preludin) and diethylpropion (Tenuate, Tepanil) in thirty-four obese diabetic patients is reported. In this investigation the purpose or possible effects of the medication used were not known to the subjects. Phenmetrazine was demonstrated to be a pharmacologically active anorexigenic substance. It produced significant weight loss in a majority of unprepared obese diabetic patients even after a preceding course of diethylpropion. The latter appeared to exert a milder anorexigenic effect which was almost completely blocked by a preceding course of phenmetrazine. Central nervous system stimulation with phenmetrazine, while not excessive, was greater than with diethylpropion. B.F.K.

Fisher, R. B.; and Young, D. A. B. (Dept. of Biochemistry, Univ. of Oxford, Oxford, England): SEASONAL CHANGES IN THE INSULIN STIMULATED EFFLUX OF GALACTOSE FROM THE ISOLATED PERFUSED RAT HEART. J. Physiol. *157*:32-34, June 1961.

The isolated rat heart was perfused with a buffered medium containing galactose in order to load the cells with this sugar. The rate of efflux of galactose was then studied during perfusion with plain buffer. Efflux during the fourth minute of washing out was accelerated by the addition of insulin to the wash fluid used for perfusion. The effect of insulin was studied at concentrations ranging from 0.2 to 2 milliunits per ml. It was observed that at the highest insulin concentrations the augmentation of efflux rate was relatively smaller, suggesting that there is a limit to the amount of insulin which can combine with muscle cells. At any given concentration of insulin, the rate of efflux was faster in summer than in winter. H.T.N.

Fordham, Christopher C., III (Dept. of Med., Univ. of North Carolina Sch. of Med., Chapel Hill, N. C.): A PROBLEM OF INSULIN DOSAGE. North Carolina M. J. *22*:259-60, June 1961.

A major problem presented by diabetic patients is that of optimal insulin dosage. Despite broad differences in viewpoint concerning the permissive versus the restrictive control of diabetes, it is generally agreed that adequate management should permit the avoidance of the symptoms of both hyperglycemia and hypoglycemia. Less widely recognized but of major importance is a further consequence of insulin hypoglycemia in the diabetic patient, that of "rebound hyperglycemia," often with mild ketoacidosis, which may occur within a few hours following hypoglycemia. Ironically, the hyperglycemia which succeeds the hypoglycemic bout is often construed by patient and physician alike as indicating the need for more, rather than less, insulin. This circumstance may well produce a widely fluctuating clinical and chemical state often described as "brittle diabetes." Though no simple and easy device for achieving the desired discrimination can here be offered, certain observations may be helpful. A symptomatic history of episodes suggestive of hypoglycemic bouts followed within hours by a tide of glycosuria is highly suggestive of hyperinsulinism. From the pattern of glycosuria, one may often obtain evidence suggestive of this phenomenon. A key finding which should alert the physician that bouts of hypoglycemia may be contributing to poor diabetes control is that of widely fluctuating urinary sugar values. The documentation of an episode of hypoglycemia by obtaining a blood sugar at the expected or the observed symptomatic time is most helpful. It may on occasion be judicious to hospitalize the patient and obtain blood-sugar observations at two-hour intervals throughout the day. W.R.K.

Ghadmi, H.; Clarke, J. T.; and Shwachman, H. (Div. of Lab. & Res. & the Dept. of Med., Children's Med. Center & Dept. of Pediat., Harvard Med. Sch., Boston, Mass.): SIMULTANEOUS SCREENING FOR MELLITURIA AND AMINOACIDURIA. Canad. M.A.J. *84*:1308-12, June 10, 1961.

A method is described for detection of as little as 20 mg. per 100 ml. of glucose and small amounts of aminoacids in urine. The screening test utilizes staining technics and when combined with unidirectional sugar chromatogram could differentiate glucose from other reducing sugars.

The authors illustrated the application of this method in twenty-one patients, both children and adults. S.B.B.

Gilgore, Sheldon G.; and Rupp, Joseph J. (Dept. of Med., & Div. of Endocrine and Cancer Res., Jefferson Med. Coll. Hosp., Philadelphia, Pa.): RESPONSE OF BLOOD GLUCOSE TO INTRAVENOUS SALICYLATE. Metabolism *10*:419-21, May 1961.

The authors studied blood glucose levels of five normal subjects and five patients with mild diabetes mellitus before, during and after two-hour intravenous infusion of 5 gm. sodium salicylate. Blood sugar levels in the normal subjects did not change significantly; whereas those of diabetics dropped an average of 28 per cent two hours after the end of the intravenous infusion, at which time the last blood specimen was taken. Since the salicylate effect is slow in onset compared with that of insulin or tolbutamide, and since it affects diabetics but not normal subjects, the blood sugar lowering effect of salicylate is probably not related to increased production or release of insulin. C.A.R.

Givner, Isadore (Univ. & Grand Central Hosp., New York Eye and Ear Infirmary, & City Hosp. at Elmhurst, New York, N. Y.): OBSERVATIONS ON THE EYE IN DIABETES. New York J. Med. *61*:2446-53, July 15, 1961.

The author discusses eye findings and problems in diabetes, with specific reference to the fundus, cataracts, retinopathy and management. C.A.R.

Goldstein, Jack E.; and Cogan, David G. (Howe Lab. of Ophthalmology, Harvard Med. Sch. and Massachusetts Eye and Ear Infirmary, Boston, Mass.): DIABETIC OPHTHALOMOPLEGIA WITH SPECIAL REFERENCE TO THE PUPIL. A.M.A. Arch. Ophth. *64*:592-600, October 1960.

In twenty-two cases of diabetic ophthalmoplegia of the third nerve, the pupil was involved in only five. Of these, four exhibited an incomplete iridoplegia and one a complete internal ophthalmoplegia. Age incidence was forty-six to seventy-four years and sex incidence was equal. Average duration of the paralysis was two and one-half months and the course was benign. In ten patients the ophthalmoplegia was the first manifestation of diabetes. Diabetes control was poor in eleven of the twenty patients at the time of paralysis. Clinical findings often included previous cranial monoplegias, such as Bell's palsy, also homolateral headache, peripheral neuropathy and diabetic retinopathy. A.R.C., JR.

Grodsky, Gerold M.; and Forsham, Peter H. (Metabolic Unit for Res. in Arthritis & Allied Diseases & the Dept. of Med., Univ. of California Sch. of Med., San Francisco, Calif.): COMPARATIVE BINDING OF BEEF AND HUMAN INSULIN TO INSULIN ANTIBODIES PRODUCED IN MAN AND GUINEA PIGS. J. Clin. Invest. *40*:799-802, May 1961.

The immunoassay for extracted human insulin yielded the same results when compared with beef insulin standards whether guinea pig or human insulin antisera were used. This indicates that such human insulin binds almost as well to human anti-beef-pork antisera as does beef insulin.

Human and pork insulin molecules have the identical amino acid structure under the disulfide bridge, and the present observations would place elsewhere the configurational reasons for antigenic differences observed between human and pork insulin. S.B.B.

Handelsman, Milton B. (State Univ. of New York Downstate Med. Center & Diabetes Clinic, Long Island Coll. Hosp., Brooklyn, N. Y.): NUTRITION IN DIABETES. New York J. Med. *61*:2094-98, June 15, 1961.

The author reviews nutritional principles in adult and juvenile diabetes, outlines current practices in dietetics and discusses the role of nutrition in diabetic complications. C.A.R.

Hartmann, Alexis F., Sr.; Wohltmann, Hulda J.; and Holowach, Jean (Washington Univ. Sch. of Med., St. Louis, Mo.): RECOGNITION AND INVESTIGATION OF HYPOGLYCEMIA. J. Pediat. *58*:864, June 1961.

Drawing on their extensive experience, the authors review the clinical picture of hypoglycemia and situations in which this may be seen. Representative case histories are given. Helpful diagnostic procedures are listed, and their interpretations are discussed. R.L.J.

Hasselblatt, A.; and Sproull, D. H. (National Inst. for Med. Res., Mill Hill, London, N.W. 7, England): HYPERGLYCAEMIA INDUCED BY DRUGS. J. Physiol. *157*:124-36, June 1961.

In unanesthetized rabbits 20 μg. of adrenalin per kilogram injected into the lateral cerebral ventricle produced hyperglycemia which was attributed to absorption of the adrenalin into the blood stream. Intraventricular injection of barbitone sodium 2 mg./kg. produced flushing of the ears, sedation, ataxia, and hyperglycemia; this was not attributable to a central action on the nervous system. Intraventricular injection of leptazol 1 mg./kg. produced a hyperglycemia of central origin which was abolished by bilateral splanchnicotomy.

In cats anesthetized with pentobarbitone sodium, a dose of adrenalin 62.5 μg./kg. did not produce hyperglycemia when injected into the lateral cerebral ventricle or intraperitoneally, but induced hyperglycemia when administered intracisternally. H.T.N.

Hayes, Donald M.; Spurr, Charles L.; Felts, John H.; and Miller, Emery C., Jr. (Dept. of Int. Med., Bowman Gray Sch. of Med. of Wake Forest Coll. & the North Carolina Baptist Hosp., Winston-Salem, N. C.): VON RECKLINGHAUSEN'S DISEASE WITH MASSIVE INTRA-ABDOMINAL TUMOR AND SPONTANEOUS HYPOGLYCEMIA: METABOLIC STUDIES BEFORE AND AFTER PERFUSION OF ABDOMINAL CAVITY WITH NITROGEN MUSTARD. Metabolism *10*:183-99, March 1961.

The authors report a patient with spontaneous hypoglycemia and a large intrahepatic tumor of mesodermal origin, the twenty-fourth such case to be recorded. The hypoglycemia did not improve after regional perfusion with large doses of nitrogen mustard, although the tumor decreased in size; however, the hypoglycemia was controlled for over three months by small doses of adrenocortical steroids. Theories on the etiology of the hypoglycemia accompanying these large mesodermal tumors include: hepatic insufficiency, reflex hyperinsulinism, sympathetic blockade, high carbohydrate consumption by tumor, secretion of insulin or insulin-like substances, and elaboration of an amino acid or similar substance causing hypoglycemia by an action unlike that of insulin. Based on their observations, the authors feel that the last three of these theories warrant further exploration. C.A.R.

Henderson, M. J.; Morgan, H. E.; and Park, C. R. (Dept. of Physiol., Vanderbilt Univ. Sch. of Medicine, Nashville, Tenn.): REGULATION OF GLUCOSE UPTAKE IN MUSCLE. IV. THE EFFECT OF HYPOPHYSECTOMY ON GLUCOSE TRANSPORT, PHOSPHORYLATION, AND INSULIN SENSITIVITY IN THE ISOLATED, PERFUSED HEART. J. Biol. Chem. *236*:273-77, February 1961.

Glucose uptake by the perfused heart from hypophysectomized rats was determined in the presence of different glucose concentrations in the perfusate. Hypophysectomy cut down glucose uptake by 30 per cent, because of diminished membrane transport. This was probably due to insulin deficiency from a reduction in growth hormone. Addition of insulin corrected transport and glucose uptake to the level found in normal muscle. A.R.C., JR.

Huggins, A. K.; Smith, M. J. H.; and Moses, V. (Dept. Chem. Path., King's College Hosp. Med. Sch., Denmark Hill, London, S.E. 5, England): UNCOUPLING REAGENTS AND METABOLISM. 3. EFFECTS OF SALICYLATE AND 2:4-DINITROPHENOL ON THE INCORPORATION OF C^{14}-LABELED PYRUVATE INTO THE SOLUBLE INTERMEDIATES OF ISOLATED RAT TISSUES. Biochem. J. 79:271-75, May 1961.

Salicylate and dinitrophenol produced similar effects on the distribution of radioactivity in intermediates of oxidative metabolism which appear to be consistent with their action in uncoupling oxidative phosphorylation. Further, salicylate increased the incorporation of labeled pyruvate into glutamic acid of liver and kidney and inhibited serum transaminase activity. J.A.

Kalliomäki, J. L.; Markkanen, T. K.; and Sourander, L. B. (Med. Clin., Univ. of Turku, Finland): CORRELATION BETWEEN INSULIN REQUIREMENT AND RENAL RETENTION IN DIABETIC NEPHROPATHY. Acta med. scandinav. *166*:423-24, May 5, 1960.

Thirty-three patients with diabetic nephropathy were studied. The average insulin requirement before the retention phase was 67 units daily. When the nonprotein nitrogen was over 71 mg. per cent or the serum creatinine over 5.0 mg. per cent the average insulin requirement decreased to 37 units daily. In the unselected control series comprising thirty-six diabetics without nephropathy the insulin requirement was 46 units daily. B.F.K.

Kaplan, Capt. Norman M. (Dept. of Med., USAF Hosp., Lackland, USAF Aerospace Med. Center, Lackland Air Force Base, Tex.): TOLBUTAMIDE TOLERANCE TEST IN CARBOHYDRATE METABOLISM EVALUATION. Arch. Int. Med. *107*:212-24, February 1961.

The clinical usefulness of the intravenous tolbutamide tolerance test as compared to glucose-loading technics has been assessed in 456 subjects. The procedure has been found to be safe, reproducible, easily performed and interpreted. The nondiabetic response is distinctly different from that of patients with overt diabetes. A significant number of persons without conditions known to affect carbohydrate metabolism and who have fasting normoglycemia but abnormal oral glucose tolerance have normal reactivity to tolbutamide. The majority of patients with advanced age, obesity, liver disease, steroid therapy, and thyrotoxicosis have normal tolbutamide tolerance even in the presence of elevated oral glucose curves. The test appears to offer adequate sensitivity and improved specificity, except during pregnancy. B.F.K.

Kellock, T. D. (Diabetic Clinic, Central Middlesex Hosp., London, England): BIRTH WEIGHT OF CHILDREN OF DIABETIC FATHERS. Lancet *1*:1252-54, June 10, 1961.

Birth weights of the children of 117 diabetic men and 117 controls of the same age distribution were obtained. The diabetics had fewer children (161) than the controls (200); but more were heavy. Thus there were twelve children weighing over ten pounds and twenty-nine over nine pounds among those of diabetic fathers, compared with four and thirteen respectively in the larger group of those of the controls. This difference is not related to differences in build in the two groups. W.R.K.

Kerkhof, Arthur C. (Minneapolis, Minn.): UNCONSCIOUSNESS WITH DIABETES. Journal-Lancet *81*:207-09, May 1961.

The causes of unconsciousness in the diabetic patient are most commonly due to (1) insulin shock, (2) diabetic acidosis and coma, and (3) peripheral vascular shock. Although the history is of prime importance in determining treatment, unfortunately many diabetic patients are found unconscious and thus unable to supply the desired information.

A summary of symptoms and therapeutic course for the various causes of unconsciousness is given. J.A.

Kinsella, Thomas J.; and Lindemaun, Charles E. (Minneapolis, Minn.): SURGICAL TREATMENT OF TUBERCULOSIS IN THE DIABETIC PATIENT. Journal-Lancet *81*:155-58, April 1961.

A review of the medical surgical management of tuberculous pulmonary disease covering eighty-three operations upon forty-nine diabetic patients is presented. The combination of diabetes and pulmonary tuberculosis, while not desirable, may be adequately managed. These patients, under proper medical control, may be successfully treated by resections or other surgical means with no greater risk than the nondiabetic patient and with equally good results. J.A.

Kirschner, Robert; and Leopold, Irving H. (Grad. Sch. of Med., Univ. of Pennsylvania and the Res. Dept. of the Wills Eye Hosp., Philadelphia, Pa.): RETINAL CHANGES IN THE ALLOXAN DIABETIC RAT MAINTAINED ON A HIGH FAT DIET: PRELIMINARY STUDIES UTILIZING A NEW FLAT RETINA TECHNIQUE. A.M.A. Arch. Ophth. *64*:681-84, November 1960.

Carotid arteries in rats were injected with 3 per cent silver nitrate, and the eyes were enucleated and fixed in 10 per cent formol-saline. Alloxanized animals fed a diet high in fat for three months exhibited areas of reticular thinning in the retinal capillaries. Microaneurysms were not found. Pathogenesis is thought to relate to altered lipid metabolism with decreased fatty acid synthesis due to lack of coenzyme TPN. A.R.C., JR.

Korhonen, Jyrki; and Koskinen, Pentti (Med. Dept. of Regional Hosp. of Central Finland, Finland): ACUTE MYOCARDIAL INFARCTION: A STUDY OF THE SEX DISTRIBUTION, IMMEDIATE MORTALITY, AGE DISTRIBUTION, INCIDENCE OF HYPERTENSION AND DIABETES, AND THE EFFECT OF THE SEASONS IN A SERIES OF 376 CASES. Ann. med. int. Fenniae *49*:247-54, 1960.

The series comprised 376 patients with myocardial infarction from 1954 to 1958 and January to September, 1959. The patients were treated with anticoagulants. The male-female ratio was 3:1. The immediate mortality rate of the patients was 23.7 per cent; 21.8 per cent of the men and 29.7 per cent of the women died. Diabetes mellitus was fairly rare (6.7 per cent of the total). Contrary to previous investigations, there was no statistical validity to show that diabetes increased the mortality rate. W.R.K.

Lambert, Thomas H.; Johnson, Roy B.; and Paul, Geoffrey R. (The Scripps Clin. & Res. Foundation, La Jolla, Calif.): GLUCOSE AND CORTISONE-GLUCOSE TOLERANCE IN NORMAL AND "PREDIABETIC" HUMANS. Ann. Int. Med. *54*:916-23, May 1961.

This is a study of the results of the oral glucose tolerance test performed alone or after the oral administration of steroids in a group of 300 active individuals divided into controls and individuals with relatives who were known diabetic patients.

No significant difference could be demonstrated between these two groups with respect to their response to the two types of glucose tolerance. S.B.B.

Leboeuf, Bernard; and Cahill, George F., Jr. (Depts. of Med., Harvard Med. Sch. and the Peter Bent Brigham Hosp. and the Baker Clin. Res. Lab., New England Deaconess Hosp., Boston, Mass.): STUDIES ON RAT ADIPOSE TISSUE IN VITRO. VIII. EFFECT OF PREPARATIONS OF PITUITARY ADRENOCORTICOTROPIC AND GROWTH HORMONES ON GLUCOSE METABOLISM. J. Biol. Chem. *236*:41-46, January 1961.

Addition of ACTH and pituitary growth hormone from sheep to epididymal adipose tissue produced an increased glucose uptake and fatty acid release. In the presence of insulin the recovery of glucose carbon in glycogen and in tissue fatty acids was depressed. Increased glucose metabolism by the Embden-Meyerhof pathway was suggested by increased metabolism of glucose carbon 1 to glyceride-glycerol, to tissue fatty acids and to CO_2 from the citric acid cycle. These hormones apparently act by increasing the accumulation of free fatty acids with a resulting acceleration of oxidation. A.R.C., JR.

Leonards, Jack R.; Landau, Bernard R.; Craig, James W.; Martin, F. I. R.; Miller, Max; and Barry, Frank M. (Depts. of Biochemistry, Med. & Surg., Sch. of Med., Western Reserve Univ., Cleveland, Ohio): SITE OF ACTION OF TOLBUTAMIDE IN THE DOG. Metabolism *10*:290-303, April 1961.

The authors injected tolbutamide (30 mg./kg. body weight) into dogs fed a high-protein or high-carbohydrate diet and prepared with catheters in their hepatic and portal veins and aortas so that hepatic glucose output could be measured directly. In some experiments, glucose-C^{14} was administered prior to tolbutamide. In other experiments, glucose was infused following tolbutamide administration to maintain normoglycemia. Tolbutamide administration to the protein-fed dog did not decrease hepatic glucose output, although a decrease or cessation in the rate of decline of circulating glucose specific activity was observed. Both an increase in the rate of removal of circulating labeled glucose and in the quantity of glucose required to maintain a constant blood glucose concentration indicated increased peripheral utilization of glucose following tolbutamide administration. In the carbohydrate-fed dog, after tolbutamide administration, there appeared to be a marked increase in peripheral utilization of glucose and also a decrease in hepatic glucose output at normoglycemic concentrations. This pattern of glucose metabolism produced by tolbutamide was similar to that observed previously with insulin. The results are in accord with the prime mode of the action of tolbutamide being the release of insulin. C.A.R.

Lewis, B.; Pilkington, T. R. E.; and Hodd, K. A. (Med. Unit, St. George's Hosp. Med. Sch., London S.W. 1, England): A MECHANISM FOR THE ACTION OF UNSATURATED FAT IN REDUCING THE SERUM CHOLESTEROL. Clin. Sc. *20*:249-54, April 1961.

Plasma lipoproteins containing C^{14}-labeled cholesterol were obtained from donor rats and injected into other rats. Bile acid and sterol excretion by the recipients increased when an unsaturated fat was fed to the donors of the lipoprotein, but were little affected when the recipients were given such a diet. Alteration of the composition of the cholesterol-bearing lipoproteins of plasma is an important mechanism by which unsaturated fat influences sterol metabolism. J.A.

Lindgren, Lars (First Dept. Med., St. Göran's Hosp., Stockholm, Sweden): ENORMOUS DOSE OF INSULIN WITH SUICIDAL INTENT. Acta med. scandinav. *167*:297-300, July 15, 1960.

A fifty-eight-year-old diabetic male, who had been taking insulin for seventeen years, took between 3200 and 3500 units of Lente, the single largest dose of insulin on record. Insulin was not required until five days after treatment which con-

sisted of continuous intravenous glucose and occasional injections of adrenalin. The patient has been under observation for eight months without the development of mental or physical complications. B.F.K.

Morgan, H. E.; Henderson, M. J.; Regen, D. M.; and Park, C. R. (Dept. of Physiol., Vanderbilt Univ. Sch. of Med., Nashville, Tenn.): REGULATION OF GLUCOSE UPTAKE IN MUSCLE. I. THE EFFECTS OF INSULIN AND ANOXIA ON GLUCOSE TRANSPORT AND PHOSPHORYLATION IN THE ISOLATED, PERFUSED HEART OF NORMAL RATS. J. Biol. Chem. *236*: 253-61, February 1961.

Hearts removed from normal rats were perfused with a warm Krebs-Henseleit bicarbonate buffer. Different amounts of insulin and glucose were added to the perfusate, and extracellular transfer was determined. The rate of sorbitol distribution in the extracellular space indicated glucose transfer. Uptake through the muscle cell membrane was limited by glucose transport. Insulin addition increased glucose uptake and phosphorylation became the major limiting step. Anoxia caused an increase in glucose uptake, further accelerated by insulin. Phosphorylation was rate-limiting at higher concentrations of glucose. A.R.C., JR.

Morgan, H. E.; Cadenas, E.; Regen, D. M.; and Park, C. R. (Dept. of Physiol., Vanderbilt Univ. Sch. of Medicine, Nashville, Tenn.): REGULATION OF GLUCOSE UPTAKE IN MUSCLE. II. RATE-LIMITING STEPS AND EFFECTS OF INSULIN AND ANOXIA IN HEART MUSCLE FROM DIABETIC RATS. J. Biol. Chem. *236*:262-68, February 1961.

The perfused diabetic heart taken from alloxanized rats exhibited a reduction in inward transport through the cell membrane which limited glucose uptake. Transport was corrected to normal by insulin, but glucose uptake was limited by phosphorylation. Anoxia stimulated transport and phosphorylation to a small degree, increasing glucose uptake. Insulin activity was retained even after production of anoxia. Restoration of oxygen reversed the effects of anoxia. A.R.C., JR.

Naide, Meyer; and Schnall, Charles (Dept. of Med., Woman's Med. Coll. of Pennsylvania, and Dept. of Med., Albert Einstein Med. Center, Philadelphia, Pa.): BONE CHANGES IN NECROSIS IN DIABETES MELLITUS: DIFFERENTIATION OF NEUROPATHIC FROM ISCHEMIC NECROSIS. Arch. Int. Med. *107*:380-83, March 1961.

The X ray is of prime importance in differentiating ischemic from neuropathic lesions in the feet of diabetic patients. Paradoxically, the prognosis of a foot with soft-tissue necrosis is much better if bone destruction or resorption is present on X ray, indicating neuropathy, than if soft-tissue necrosis is present in an ischemic foot with the bone intact. The explanation for the better prognosis in the patient with the neuropathic bone lesion is that for rarefaction, lysis, or destruction of bone to occur there must be blood flowing through it. If blood supply is severely reduced bone will not undergo destruction or lysis. B.F.K.

Paloyan, Edward; and Harper, Paul Vincent, Jr. (Dept. of Surg., The Univ. of Chicago, Chicago, Ill.): GLUCAGON AS A REGULATING FACTOR OF PLASMA LIPIDS. Metabolism *10*: 315-23, April 1961.

The islets of Langerhans in the body and tail of the pancreas of the dog contain alpha cells, whereas in the uncinate process and the part of the gland drained by the duct of Wirsung, islets are largely devoid of these cells. In eighteen dogs, surgical removal of the portions of the gland containing alpha cells resulted in a marked hyperlipemia when a small remnant of pancreas was left to supply pancreatic juice to the bowel. No such hyperlipemia occurred when the remnant contained significant numbers of alpha cells. The hyperlipemia was abolished by the daily intramuscular injection of 10 mg. of crystalline glucagon in oil. These results support the hypotheses that glucagon originates solely from the pancreatic alpha cells, that the observed hyperlipemia was a manifestation of glucagon deficiency, and that glucagon is an important factor in regulating blood lipids. C.A.R.

Penick, S. B.; and Hinkle, L. E., Jr. (New York Hosp., Cornell Medical Center, New York, N. Y.): DEPRESSION OF FOOD INTAKE INDUCED IN HEALTHY SUBJECTS BY GLUCAGON. New England J. Med. *264*:893, May 4, 1961.

With the use of a random double blind procedure, 110 acute experiments were carried out with eight healthy medical students to compare the effects of glucose 100 gm. by mouth, glucagon 1 mg. intramuscularly and a placebo upon food intake. Neither glucose nor glucagon had any significant effect upon the food intake of the subjects half an hour after administration despite the fact that both produced significant elevations in the absolute blood glucose concentration and in the arterial venous blood glucose differences at such times. Glucagon, but not glucose or placebo, produced a significant reduction in the food intake at test meals. This effect was delayed beginning one hour after administration reaching a peak at two to three hours and continuing for five hours. The mean depression of caloric intake was 30 per cent below the mean control level. The administration of glucagon occasionally caused moderate nausea and some complaints of anorexia but there was no consistent relation between the occurrence of these symptoms and the depression of food intake. S.S.

Pfeiffer, E. F.; Ditschuneit, H.; and Ziegler, R. (I. Med. Dept., Univ. Frankfurt a.M., Germany): ON THE BLOOD INSULIN ASSAY WITH THE RAT EPIDIDYMAL FAT PAD USING "TAGGED" GLUCOSE. IV. THE MECHANISM OF INSULIN SECRETION IN HEALTH AND IN MATURITY ONSET DIABETES AFTER REPEATED LOADING WITH GLUCOSE, ARYL SULFONYLUREA AND HUMAN GROWTH HORMONE: A CONTRIBUTION TO THE PROBLEM OF THE ETIOLOGY OF THE MATURITY ONSET DIABETES. Klin. Wchnschr. *39*:415-26, April 15, 1961.

The rate of oxidation of 1-C^{14}-glucose to $C^{14}O_2$ in the isolated epididymal fat pad of the rat under the influence of insulin was used as a method for the estimation of serum insulin activity. The method has been described in detail in earlier publications of the authors (Klin. Wchnschr. *37*:1234, 1959). An attempt was made to determine the functional capacity of the insulin producing islet cell system in maturity onset diabetes by simultaneous estimations of alterations of the blood sugar level and serum insulin activity under various stresses. Repeated administrations of glucose or arylsulfonylureas and of human growth hormone were employed as such stresses. Serial studies were carried out on thirteen patients with maturity onset diabetes and seven healthy individuals. Repeated glucose loads (2 x 50 gm. glucose i.v., 5 x 50 gm. glucose orally during twenty-four hours), produced in the maturity onset diabetes little or no increase in serum insulin

activity, while good responses were obtained in the control individuals. Likewise, maturity diabetes was much more refractory to repeated sulfonylurea administration (25 mg./kg. i.v. five times during twenty-four hours) than the controls. Healthy individuals showed an increase of serum insulin activity about four hours after the first injection of tolbutamide, while the diabetic serum showed a new increase of insulin activity after a refractory period of as much as twenty-four hours. The immediate response to human growth hormone was almost identical in the diabetic and the normal serum. The increased serum insulin activity of the controls persisted, however, far longer than that of the diabetics. The authors concluded that the maturity onset diabetes is characterized by a localized endocrine deficiency of the islet cell system. While in growth onset diabetes this endocrine defect is manifest under normal conditions, the hormonal deficiency of the maturity onset disease can be demonstrated only if the islet cell system is put under stress. The defect is characterized both by an inadequate adaptation of insulin secretion (response to glucose or to sulfonylureas) and by an inadequate production of insulin (response to human growth hormone). M.G.G.

Post, R. L.; Morgan, H. E.; and Park, C. R. (Dept. of Physiol., Vanderbilt Univ. Sch. of Med., Nashville, Tenn.): REGULATION OF GLUCOSE UPTAKE IN MUSCLE. III. THE INTERACTION OF MEMBRANE TRANSPORT AND PHOSPHORYLATION IN THE CONTROL OF GLUCOSE UPTAKE. J. Biol. Chem. 236:269-72, February 1961.

The kinetics of glucose uptake by heart muscle, under control of transport of intracellular phosphorylation, are analyzed. Graphs are given on which predicted and observed values for glucose uptake and internal glucose can be plotted. This permits an estimation of constants for transport in the normal and diabetic muscle. A.R.C., JR.

Ricketts, H. T.; Danowski, T. S.; and Lukens, F. D. W. (Chicago, Pittsburgh and Philadelphia): A PANEL DISCUSSION ON DIABETES. Maryland State Med. J. 10:169, April 1961.

This is a comprehensive panel discussion touching on such considerations as prediabetes, prevention of diabetic complications, oral therapy and management of unstable diabetes. S.S.

Rifkin, Harold; and Leiter, Louis (Med. Div., Montefiore Hosp., New York, N. Y.): DIABETES MELLITUS: DIABETIC MICROANGIOPATHY. New York J. Med. 61:1920-27, June 1, 1961.

The authors review the problem of diabetic microangiopathy complicating the course of diabetes mellitus. The specificity of diabetic microangiopathy, its pathogenesis, associated clinical features and treatment are reviewed. C.A.R.

Schlesinger, F. G.; Schwarz, F.; and Wagenvoort, C. A. (Med. Dept., Univ. Hosp., and Pathological Inst., State Univ., Utrecht, Holland): THE ASSOCIATION OF DIABETES MELLITUS WITH PRIMARY CARCINOMA OF THE PANCREAS. Acta med. scandinav. 166:337-41, April 20, 1960.

Seven cases of diabetes with carcinoma of the pancreas are described. In five patients diabetes had appeared less than two years before the onset of abdominal pain, weight loss and other symptoms of the carcinoma. The family history for diabetes was negative in four patients. In four cases the islands of Langerhans were studied and showed no abnormalities. In one, the alpha-beta cell ratio was determined and appeared normal. Extensive fibrosis appeared to be related to the onset of diabetes, in pancreatic carcinoma. It is concluded that a diabetic glucose tolerance curve should be made in middle-aged patients with intractable abdominal pain. B.F.K.

Shelley, Thomas F.; Frawley, Thomas F.; and Margulies, Edward J. (Sub-Dept. of Endocrinology & Metabolism, Dept. of Med. & Dept. of Surg., Albany Med. Coll. of Union Univ., Albany, N. Y.): STUDIES ON THE MECHANISMS OF TOLBUTAMIDE HYPOGLYCEMIA. Metabolism 10:275-89, April 1961.

Effects of intravenous sodium tolbutamide injection (50-100 mg./kg.) and intravenous insulin injection (0.2 u./kg.) or infusion (0.33 u./kg.) on peripheral glucose uptake were compared in dogs with portacaval shunts and hepatectomized dogs. The onset of tolbutamide hypoglycemia in portacaval shunt dogs was not accompanied by an increased peripheral glucose uptake as measured by arteriovenous glucose differences in the leg. In the absence of the liver, however, the onset of tolbutamide-induced hypoglycemia was caused by an increase in peripheral glucose uptake. These results are interpreted as indicating that, in the dogs with portacaval shunts, the presence of the liver modifies the hypoglycemic response to tolbutamide so that hepatic rather than peripheral glucose uptake increases. Furthermore, since the onset and characteristics of tolbutamide-induced hypoglycemia in the absence of the liver resembled that produced by insulin in the intact or portacaval shunt animal, it is presumably mediated by tolbutamide-induced secretion of endogenous insulin. C.A.R.

Singh, Inder; Sehra, K. B.; and Bhargava, S. P. (Armed Forces Med. Coll., Poona, and Irwin Hosp., New Delhi, India): TOLBUTAMIDE IN CIRRHOSIS OF THE LIVER. Lancet 1:1144-46, May 27, 1961.

The increase in the glycogen content of the liver under the influence of tolbutamide therapy, first reported by German workers in 1956, led the authors to believe that tolbutamide may protect the liver from various types of injury and may even improve its function. The increase in serum proteins appears due to some specific effect of tolbutamide on the liver. The increased appetite following tolbutamide therapy and better nutrition must have contributed towards higher intake of proteins in food and to some extent in their synthesis into serum proteins in our cases; but the effect without tolbutamide was never so significant as with tolbutamide. Further, withdrawal of tolbutamide reversed the clinical improvement. Some explanation may be found in reports on the effect of tolbutamide on amino acids. Thus, pretreatment of rats with tolbutamide has been observed to increase the incorporation of C^{14}-glycine into protein in liver slices by Recant and Fischer (1957); and DeMeutter et al. (1958) have noticed that tolbutamide injected intravenously into human subjects decreases serum-α-aminoacids. W.R.K.

Skillman, Thomas A.; Hamwi, George J.; Driskil, Helen; and Penrose, Margaret H. (Ohio State University, Columbus, Ohio): ORAL HYPOGLYCEMIC THERAPY: LONG TERM RESULTS IN OLDER DIABETIC PATIENTS. Geriatrics, May 1961.

An account is given of 125 diabetic patients over age sixty who were treated with tolbutamide or chlorpropamide and observed in an outpatient research clinic for periods of three

to forty-eight months. In all patients control of hyperglycemia could not be affected by diet alone. Eighty members of this group were treated with 1 to 2 gm. of tolbutamide a day, with 62.5 per cent optimal response within thirty days of initiation of therapy. However, over a twenty-four-month observation period, approximately 2.5 per cent of the responsive patients failed during each month of continued treatment. When seventy patients were treated with chlorpropamide and daily doses of 0.1 to 0.5 gm., 57 per cent responded optimally within thirty days and the rate of secondary failure was approximately 1 per cent monthly. One third of the patients who were primary or secondary failures with tolbutamide were found to be responsive when treated with chlorpropamide. The probability, or primary or secondary response, or failure, could not be predicted by knowledge of age, sex, weight or duration of the diabetic state. Treatment with the oral agents did not appear to influence body weight. In long-term treated patients a minor trend towards elevation of the mean value of serum cholesterol was noted. No evidences of severe toxicity were noted with either agent during 1,731 patient months of observation. S.S.

Walker, Geoffrey; and Kinsell, Laurance W. (Inst. for Metabolic Res., Highland-Alameda County Hosp., Oakland, Calif.): CLINICAL EXPERIENCE WITH CHLORPROPAMIDE. California Med. 94:344-49, June 1961.

Seventy-eight patients with mild diabetes were treated with chlorpropamide in doses up to 1 gm. daily. Eight showed primary failure to respond and two showed an initial but not a sustained response. Patients whose diabetes was of recent onset and who had not been treated with insulin or a sulfonylurea required smaller doses than the remainder, except for a group of patients who had been diabetic for more than fifteen years. The average dose for the sixty-four patients who took chlorpropamide for more than three months was 0.46 gm. per day. Serious side effects were uncommon and disappeared when the drug was stopped. One patient became jaundiced, one had a rash, and one showed granulocytopenia. Changes in liver function tests were seen in seventeen out of fifty-six patients treated for more than three months and were correlated with the dosage of the drug and the duration of treatment. W.R.K.

Weil, William B., Jr.; and Sussman, Marvin B. (Dept. of Pediatrics, Dept. of Sociology, Western Reserve University, Cleveland, Ohio): BEHAVIOR, DIET AND GLUCOSURIA OF DIABETIC CHILDREN IN A SUMMER CAMP. Pediatrics 27:118-27, January 1961.

Observations were made on thirteen preadolescent diabetic boys at a summer camp. Glucosuria, hypoglycemia, dietary intake with self-regulated and prescribed diets, emotional adjustment and measures of group structure were recorded and correlated to determine the relationship between psychologic-sociologic factors and metabolic changes in the diabetic state. Average values for glucosuria for the entire group indicated that during the period of a prescribed diet, the percentage of glucose in the urine was not statistically different during any of the three periods of the twenty-four hours. With the self-regulated diet there was a significantly greater percentage of glucose in the urine during the day and evening periods than during the night period. Well-adjusted boys had less of an increase in glucosuria when they went from a prescribed diet to a self-regulated one than did the poorly adjusted boys. The differences in glucosuria were partially correlated with the differences in caloric consumption, the poor adjustors having a greater increase in calories consumed than the good adjustors. W.R.K.

Werk, Emile E., Jr.; Garber, Stanley; and Sholiton, Leon J. (Dept. of Med., Veterans Administration Hosp. and the Metabolism Lab., Univ. of Cincinnati Coll. of Med., Cincinnati, Ohio): EFFECT OF SYMPATHETIC BLOCKADE ON CHANGES IN BLOOD KETONES AND NONESTERIFIED FATTY ACIDS FOLLOWING HYPOGLYCEMIA IN MAN. Metabolism 10: 115-25, February 1961.

Levels of circulating ketones, nonesterified fatty acids and 17-hydroxycorticosteroids were determined following acute hypoglycemia induced by intravenously administered glucagon-free insulin in patients with and without autonomic blockade by hexamethonium. A significant rise in concentration of blood ketones and plasma nonesterified fatty acids above the control values occurred in both groups following the hypoglycemia. However, the increases in both substances were slightly but significantly delayed in the patients treated with hexamethonium. The response of plasma 17-hydroxycorticosteroids to the hypoglycemia was not diminished by the autonomic blockage, which is consistent with the possibility that cortisol may be a contributing factor in the above changes in lipid metabolism. It is suggested that these hypoglycemia-induced changes in lipid metabolism can occur without the action of the sympathetic nervous system, but in the intact individual sympathetic activity may serve as an early initiating factor promoting lipid mobilization and hepatic ketogenesis. C.A.R.

Wheelock, Frank C., Jr. (Massachusetts General Hospital, Boston, Mass.): TRANS-METATARSAL AMPUTATIONS AND ARTERIAL SURGERY IN DIABETIC PATIENTS. New Eng. J. Med. 264: 316, Feb. 16, 1961.

Experiences with transmetatarsal amputations in diabetic patients with peripheral gangrene are discussed. Certain requirements must be met if the procedure is to be successful, namely, a distally located lesion localized in a foot with adequate collateral circulation. Four hundred and twenty-eight such operations were performed and 63 per cent were healed two or more years later. At the end of five years, 46 per cent were still satisfactory.

An additional feature of this article includes a discussion of arterial reconstruction in diabetic patients with incapacitating claudication, ischemic rest pain or gangrene. These patients must be carefully screened in the hope of finding those individuals with a localized arterial block suitable for arterial reconstruction. However, the diffuseness of their arteriosclerosis limits this procedure in many diabetic patients. Of sixty-five patients operated upon, fifty-seven were initially successful and thirty-six reconstructions remained open, either until the patients' subsequent death or to the present time.

The transmetatarsal amputation for distal gangrene of the foot has proved a valuable procedure and one responsible for the salvage of many legs. Arterial reconstruction of the diabetic patient is worth-while with segmental occlusions in the aortic, iliac, femoral or upper popliteal arteries. S.S.

ORGANIZATION SECTION

OFFICERS AND MEMBERS OF COUNCIL, AMERICAN DIABETES ASSOCIATION, 1961-1962

HONORARY PRESIDENTS, ELLIOTT P. JOSLIN, M.D., *Boston*; CHARLES H. BEST, M.D., *Toronto*

PRESIDENT
BLAIR HOLCOMB, M.D., *Portland, Oregon*

SECRETARY
LAURENTIUS O. UNDERDAHL, M.D., *Rochester, Minnesota*

FIRST VICE PRESIDENT
JEROME W. CONN, M.D., *Ann Arbor*

TREASURER
EDWIN W. GATES, M.D., *Niagara Falls*

SECOND VICE PRESIDENT
THOMAS P. SHARKEY, M.D., *Dayton*

EXECUTIVE DIRECTOR
J. RICHARD CONNELLY, *New York*

MEMBERS OF COUNCIL

TERM EXPIRING 1962

LOUIS K. ALPERT, M.D., *Washington, D.C.*
WILLIAM R. JORDAN, M.D., *Richmond*
HARVEY C. KNOWLES, JR., M.D., *Cincinnati*
ARNOLD LAZAROW, M.D., *Minneapolis*
ALBERT E. RENOLD, M.D., *Boston*
EDWIN L. RIPPY, M.D., *Dallas*

TERM EXPIRING 1963

THADDEUS S. DANOWSKI, M.D., *Pittsburgh*
WILLIAM H. GRISHAW, M.D., *Beverly Hills*
GEORGE J. HAMWI, M.D., *Columbus, Ohio*
HENRY E. MARKS, M.D., *New York*
HENRY E. OPPENHEIMER, M.D., *St. Louis*
PRISCILLA WHITE, M.D., *Boston*

TERM EXPIRING 1964

JOSEPH H. CRAMPTON, M.D., *Seattle*
LEO GOODMAN, M.D., *Fresno*
ROBERT C. HARDIN, M.D., *Iowa City*
EDGAR A. HAUNZ, M.D., *Grand Forks*
RACHMIEL LEVINE, M.D., *New York*
CHRISTOPHER J. MCLOUGHLIN, M.D., *Atlanta*

PAST PRESIDENTS

ALEXANDER MARBLE, M.D., *Boston*
FRANCIS D. W. LUKENS, M.D., *Philadelphia;* FRANKLIN B. PECK, SR., M.D., *Monticello, Indiana*

ASSEMBLY OF STATE GOVERNORS AND AFFILIATE DELEGATES

MAURICE PROTAS, M.D., Chairman, *Washington, D.C.;* JAMES B. HURD, M.D., Vice Chairman, *Chicago*
W. BERNARD YEGGE, M.D. (1962), *Denver;* ELTON R. BLAISDELL, M.D. (1963), *Portland, Maine*
CARLISLE MORSE, M.D. (1964), *Louisville*

Twenty-first Annual Meeting

The Twenty-first Annual Meeting of the American Diabetes Association was held at the Hotel Commodore in New York City, June 24-25, 1961. Names of Officers and Councilors elected for the 1961-62 organizational year, as announced in the July-August DIABETES, are listed on the masthead above.

SCIENTIFIC SESSIONS

Five Scientific Sessions were held this year: Saturday, June 24, morning and afternoon; Sunday, June 25, two concurrent morning sessions and one in the afternoon. In all, thirty-seven papers were presented and twenty-eight others were included in the printed program by title.

The Committee on Scientific Programs, under the Chairmanship of Jerome W. Conn, M.D., Ann Arbor, included the following members: Blair Holcomb, M.D., Portland, Oregon, Vice Chairman; James Ashmore, Ph.D., Indianapolis; Thaddeus S. Danowski, M.D., Pittsburgh; Irving Graef, M.D., New York; and Harvey C. Knowles, Jr., M.D., Cincinnati.

The Banting Memorial Lecture was delivered by Rachmiel Levine, M.D., New York, Chairman and Professor of the Department of Medicine at New York Medical College and Director of Medical Services at Flower and Metropolitan Hospitals. His Lecture was entitled "Concerning Mechanisms of Insulin Action," and was presented at the Scientific Session on Saturday morning, June 24.

Rosalyn S. Yalow, Ph.D., recipient of the 1961 Lilly Award, presented a paper entitled "Immunoassay of Insulin," written in collaboration with Solomon A. Berson, M.D.

ANNUAL SESSION OF DELEGATES

The Eighth Annual Session of the Assembly of Delegates, now reconstituted as the Assembly of State Governors and Affiliate Delegates, was held on June 24. Maurice Protas, M.D., Washington, D.C., was re-elected Chairman, and James B. Hurd, M.D., Chicago, was elected Vice Chairman. Mrs. Charles W. McNeely, Indianapolis, was named Secretary. In addition to the usual matters considered at the Annual Session, the activities of some of the committees of the American Diabetes Association were outlined by their chairmen.

Special Sessions

These events were featured at the morning session on June 24:

- "What the Future Holds for Diabetics," a panel discussion presented by three Past Presidents of the American Diabetes Association: Randall G. Sprague, M.D., Rochester, Minnesota, Moderator; Francis D. W. Lukens, M.D., Philadelphia; and Alexander Marble, M.D., Boston.
- "Quackery in Diabetes," a paper by Oliver Field, LL.B., Director, Department of Investigation, American Medical Association.
- "Technics of Program Planning," a panel discussion with Henry E. Marks, M.D., New York, Moderator. Panelists were Abraham Bluestein, New York; L. Lewis Pennock, M.D., Pittsburgh; Mrs. Jack Van Der Beek, Bradenton, Florida; Henry E. Oppenheimer, M.D., St. Louis; and

Mr. Frank H. Jenne, Detroit.

- "Diabetes and Its Long-range Control," a professional education film of the New Jersey State Department of Health, had its premiere showing.
- "What Makes a Local Diabetes Drive Successful?" a panel discussion with Kermit E. Osserman, M.D., New York, as Moderator; and Leo Goodman, M.D., Fresno; John A. Reed, M.D., Washington, D.C.; Mrs. Charles H. Misegades, St. Louis; Mrs. Carolyn Spiegel, Chicago; and Mrs. Charles W. McNeely, Indianapolis, as panelists.

On Sunday, June 25, a session entitled "Workshop for Administrative Personnel of Affiliate Associations" was held. It opened with informal discussions of "Workable Ideas for Better Programs." Mr. Clark H. Spitler, of Denver, led the discussion on "Detection Drives"; Mr. Herman Winkelmann, of St. Louis, spoke on "Dinners for Diabetics"; Mary Youngken, R.N., of Bethlehem, Pennsylvania, described "Schools for Diabetics"; and Mrs. Clarence E. Taylor, of Pittsburgh, outlined "The Role of the Affiliate in Community Affairs." Next came an informative talk on "How to Make the Best Use of Radio and TV" by Mr. Robert Warner of New York. In the afternoon an Audio Studio was held, at which Public Education radio recordings were heard.

ANNUAL BANQUET

The Banquet was held on Saturday evening, June 24. Invocation was offered by Deaconess Maude Behrman, Director of The Mercer Memorial House, Atlantic City, New Jersey.

The Banting Medal

The Banting Medal was presented by Franklin B. Peck, Sr., M.D., President, to Dr. Rachmiel Levine, the Banting Memorial Lecturer. Francis D. W. Lukens, M.D., immediate past President, presented the Banting Medal to Dr. Peck for his services as President of the ADA.

Other Banquet Highlights

Anniversaries:

- *40th Discovery of Insulin*: Charles H. Best, M.D., D.Sc., F.R.S., of Toronto, was a guest of honor at the Banquet. Speaking informally, he recalled the days when he and Dr. Frederick G. Banting first demonstrated that insulin would work to guard the health of human diabetics. It was forty years ago this year that the two investigators discovered insulin.
- *30th New York Diabetes Association*: Irving Graef, M.D., President of NYDA and Editor of DIABETES, spoke of the founding of the New York Diabetes Association. In tracing its growth, he mentioned in particular the scientific symposia presented by its Clinical Society, and the work with diabetic children at Camp NYDA.
- *21st American Diabetes Association*: Cecil Striker, M.D., Cincinnati, first President of the American Diabetes Association and its Secretary for six consecutive years thereafter, briefly related the early history of the Association.

- Dr. Ralph West Robey, Economic Adviser of the National Association of Manufacturers, discussed the complexities of "The Washington Scene and Money."
- Mrs. Carolyn B. Spiegel, Chicago, was presented the 1961 "Citation as an Outstanding Layman." Mrs. Spiegel is one of the charter members of the Service Unit (Lay Society) of the Chicago Diabetes Association, and has served as its Chairman since 1955. Activities which Mrs. Spiegel helped direct include a cooperative program with local Health Fairs, benefits, lay meetings and the annual Diabetes Detection Drive. With her assistance, the Chicago Drive has expanded to the point where, in 1960, it included the participation of seventy-three hospitals and testing centers. Besides these activities, Mrs. Spiegel is a member of the Committee for the Summer Camp for Diabetic Children at Lake Geneva, Wisconsin.

Dr. Rachmiel Levine, Banting Memorial Lecturer, receives Banting Medal from Dr. Franklin B. Peck, Sr., President of the American Diabetes Association.

The Lilly Award

The 1961 Lilly Award was presented by Dr. Peck to Rosalyn S. Yalow, Ph.D. Dr. Yalow is Assistant Chief of the Radioisotope Service, Veterans Administration Hospital, Bronx, N.Y. The award consists of $1,000 and a medal. Its recipient is chosen by the ADA Committee on Scientific Awards from investigators under forty years of age who have been nominated by members of the Association. Its purpose is to recognize demonstrated research in the field of diabetes, taking into consideration independence of thought and originality.

Dr. Yalow has pioneered since 1947 in using radioisotopes in medical tracer work. Her research has been conducted in collaboration with Dr. Solomon A. Berson, a previous winner of the Lilly Award. Dr. Yalow, pictured on page 411, has been a key figure in the development of a sensitive technic for determination of insulin in biological materials.

Dr. Charles H. Best, guest of honor at the Annual Banquet, spoke informally to commemorate the 40th Anniversary of the discovery of insulin.

ANNUAL BUSINESS MEETING

The Annual Business Meeting was held Sunday, June 25, at 2 p.m. Dr. Peck presided and delivered the Annual Address of the President. Reports by the Secretary, Treasurer, Executive Director and Nominating Committee were followed by Installation of Incoming President, and Remarks of the newly installed President.

WHAT'S THE MATTER WITH BEING UNIQUE?

FRANKLIN B. PECK, SR., M.D., INDIANAPOLIS

On this occasion when the American Diabetes Association is celebrating its twenty-first birthday it seems fitting to pause for a moment of contemplation—about where we are, where we have been, and where we seem to be going. Presidential addresses have almost always indicated that we face some fork in the road of progress where we must choose wisely between dark oblivion and the light of eternal happiness. Thus far, the Association appears to have chosen the right path for it has grown like a well-rooted tree albeit some shaded branches seem stunted in comparison with those facing the sunshine of the contributor's dollar specially earmarked for research. The general fund, unearmarked, must carry the weight of our fourfold program of Professional Education, Public Education and Case Finding, Patient Education, and the entire overhead of the Research program. The American Diabetes Association alone, as far as I am aware, is the only organization in the health field which can boast that every dollar which is contributed for research is devoted solely to that purpose with no administrative overhead.

Bear in mind too, that the general fund, which is the backbone of our program, is still only 60 per cent self-supporting in spite of the over-all favorable increase in total funds collected. The remaining 40 per cent of income is subject to the hazards of every economic factor and whim influencing the giving of voluntary donors, be they patients, friends and families of diabetics, or the larger contributions of Sustaining Corporate Members from industry, life insurance companies, or foundations. As long as this situation exists and a large portion of income is earmarked for special projects, the organization faces the possibility of starving in the midst of plenty. A general decline in business productivity places our program in special jeopardy. We sorely need a broader base of long-term financial support to insure the fulfillment of our primary objective—to improve the lot of the diabetic. *This, then, is where we are.*

Where have we been requires only passing mention. This Association was formed as a medical society of doctors who felt collective as well as individual responsibility for the welfare of the diabetic. Willy-nilly, the projection of a professional society's activities into the public sphere immediately categorized it as a voluntary health agency. Without exception all other such organizations have been and are dominated by public-spirited laymen of good intent whose major function has been to implement the public health program of their organization by raising the vast sums necessary to support health activities on a national scale.

Experts in this field have repeatedly told us with unanimous agreement that such a program as that of the American Diabetes Association was and is impossible, irrational, schizophrenic, and obviously doomed to failure, unless accompanied by national participation in public fund raising. Nevertheless, we have persisted in this unique and ill-advised course,

Dr. Cecil Striker, first President, briefly related the early history of the American Diabetes Association on the occasion of its 21st Anniversary.

and the American Diabetes Association has remained a professional organization which has attained authoritative world leadership in its field.

But we are nonconformists in a world of changing technological culture which is conditioned to react adversely to any deviation from standard behavior.[1] We are conditioned by our mass educational system, the standardized training of our young men and women in the military, by mass communication such as radio, television, entertainment, the press, and a continuous barrage of advertising media. Departures from the behavior norm create public suspicion of being queer. Actually the "publicity weapon has become more potent than the enforcement powers of law and there is sometimes no judicial protection against it."[2] On the other hand, there are still some rugged individualists about, some of whom have actually supported us because we are unique.

And so—*where are we going?* John Millis[3] writing on "Responsibility—the Price of Authority" points out that "the most vexing problem, both for free individuals and for voluntary organizations is coping with change" and that "The voluntary organization which has sought and accepted leadership must cope with change or face the certainty that someone else, either government or another group, will do it." This is the greatest responsibility of leadership, and leaders can only come from membership. You, the members of the American Diabetes Association, must determine, and soon, whether uniqueness is a virtue, and your will must determine where we are going.

[1] Ruesch, Jurgen: Conformance, Originality, and Responsibility in Higher Education. J. Med. Educ. *34*:571, 1959.

[2] FDC Reports, Nov. 16, 1959.

[3] Millis, John: J.A.M.A. *174*:63, Sept. 3, 1960.

FRANKLIN B. PECK, SR., M.D., PRESIDENT, 1960-61

ROSALYN S. YALOW, PH.D.
Winner of the 1961 Lilly Award

Report of the Secretary

Mr. President, Members of the Association: It is extremely gratifying to me as your Secretary, as I know it is to all of our membership, that the Association is continuing to fulfill its vital objectives and provide its essential leadership in the field of diabetes.

The Twenty-second Annual Meeting will be held at The Conrad Hilton hotel in Chicago on June 23-24, 1962, immediately preceding the annual session of the American Medical Association the week beginning June 25.

The Tenth Postgraduate Course will be held in Detroit, Michigan, on Jan. 17 to 19, 1962. The first and third days of the Course (Jan. 17 and 19) will be at The Statler Hilton hotel in Detroit. The second day (Jan. 18) will be at the University of Michigan in Ann Arbor.

The Association's scientific exhibit, "Everyday Problems in Diabetes: 201 Crystal Clear Answers," is again being displayed at the American Medical Association meeting, June 25-30 in New York City. It is hoped that everyone will visit the exhibit which will be in Booth No. 2334, third floor, Scientific Exhibit Section, New York Coliseum. The exhibit was developed by the Committee on Scientific Exhibits, now under the chairmanship of Dr. C. J. O'Donovan, in collaboration with the Steering Committee of the Committee on Professional Education, chaired by Dr. T. S. Danowski. Since this exhibit was first displayed in Miami Beach last

June, the national office has received many requests for copies of the text.

The Research and Fellowships program of the Association has continued to progress and the following Fellowships have been awarded for the 1961-62 academic year:

John A. Colwell, M.D., Department of Medicine, Northwestern University Medical School, Chicago, Illinois, to continue to work with Arthur R. Colwell, Sr., M.D.

Jack Kane Goldman, M.D., Harvard University Medical School, Boston, Massachusetts, to continue to work with George W. Thorn, M.D., and Albert E. Renold, M.D.

James C. Barrett, M.D., University of Alabama Medical Center, Birmingham, to work with S. R. Hill, Jr., M.D.

John K. Davidson, III, M.D., Charles H. Best Research Institute, Toronto, Canada, to work with Reginald E. Haist, M.D., Ph.D.

Philip Levy, M.D., Guy's Hospital, London, to work with W. J. H. Butterfield, M.D.

Jesse Roth, M.D., Veterans Administration Hospital, New York, to work with Solomon A. Berson, M.D.

The Committee on Scientific Awards has selected Thomas F. Necheles of the University of Chicago as the winner of the $250 award for the best paper in the 1960-61 Graduate and Medical Student-Intern Essay Contests reporting original work. The title of his paper is "Some in Vitro Effects of Insulin and Thyroxine on Protein and Nucleic Acid Synthesis in Rabbit Bone Marrow."

The $100 prize for the best review article or case report will be awarded to Richard J. Mahler, M.D., of New York Medical College, for his paper entitled "The Relationship of Fatty Acid Metabolism to Diabetes Mellitus." Honorable mention in this prize category is being given to Roland K. Molinet, M.D., of New York Medical College, for his paper entitled "The Relationship of Glomerulosclerosis to Primary (Familial) and Secondary Diabetes."

The total membership of our Association is now 2,603 with seventeen applications pending.

Our patient education material, in particular the ADA FORECAST, with a circulation of more than 50,000, continues as one of the major service activities of the Association.

I am sorry that in the interest of time it is not possible to present a detailed report of the progress of our various committees and boards. Programs in existence as well as new projects which have been authorized are at an all-time high.

On behalf of the membership, I wish to express our appreciation to Mr. Connelly and his staff for the management of our work in the field of diabetes.

This, our Twenty-first Annual Meeting, has been a productive and informative session. I submit this, my report as Secretary of the American Diabetes Association, with my gratitude to the committees and boards which have cooperated so wholeheartedly in the carrying out of my duties.

E. PAUL SHERIDAN, M.D.

Report of the Treasurer

It is my pleasure to report that the American Diabetes Association completed the fiscal year ending March 31, 1961, with an excess of income over operating expenses (exclusive of Government Grants and Bequests) of $6,000.

Total operating income for the year amounted to $448,000 and program expenditures were $442,000.

In addition to income from earnings and contributions, which are considered as operating income, $3,700 was received from Bequests, $21,300 from the National Institutes of Health in support of the Comprehensive Survey of Diabetes Abstracts Coverage project. Including Bequests and the Government Grant, which amount to $25,000, *total income from all sources was $473,000 with $35,300 of that amount restricted to research.*

Of our *total operating income* of $448,000, 60 per cent or $268,000 is considered earned income and was received from such sources as membership dues, $14,500; DIABETES Journal subscriptions, $56,000; ADA FORECAST subscriptions, $97,000; sales of testing materials, $28,000; interest earned on savings accounts, $21,600; interest and dividends on investments, $6,900; and sales of miscellaneous publications, discounts on purchases, etc., amounting to $36,500. The Ninth Postgraduate Course added another $7,500.

The remaining *40 per cent of total income or $180,000 was received from contributions.* A breakdown of the major sources of contributions is as follows: Active and Associate Members contributed $1,000; Corporate Members, $1,250; Contributing Corporate Members, $1,000; Sustaining Corporate Members, $136,750; Affiliate Associations, $7,550; Foundations, $14,800; ADA FORECAST subscribers, $9,300; In Memoriam Gifts, $5,000.

As our list of contributors increases each year, time prevents me from acknowledging individual gifts. It gives me great pleasure, however, to express appreciation to all who have contributed to the support of our program.

At this time, I would like to mention the remarkable growth of The Nordisk Insulin-fond Foundation for the Elliott P. Joslin Fellowship. This fund, which is carried on our books at cost or about $72,000 was valued at $146,000 based on the market value as of March 31, 1961.

Our program expenditures for the past fiscal year amounted to $442,000. Of this amount, $125,900 or 29 per cent was spent on Professional Education; $141,000 or 32 per cent was spent on Patient Education; our Public Education and Detection program cost us $103,000 or 23 per cent; $29,475 or 7 per cent was spent on Research and Fellowships; and the remaining 8 per cent represents General Operating and Administrative costs, including the amount chargeable to depreciation of equipment.

In summarization, although the American Diabetes Association is in a sound financial position, we must continually seek increased financial support through contributions and income-producing activities to enable us to meet the requirements of an expanding program out of current income. Only in this manner can we build up sufficient reserve funds to insure more than a year's operation in the event of an emergency.

THOMAS P. SHARKEY, M.D.

Report of the Executive Director

Dr. Peck, Officers, Councilors and Members of the American Diabetes Association: As with last year, the activities of our organization have continued to grow with the growth of our educational programs, both professional and lay.

Although it may sound trite, I would suggest that with our official coming of age, our twenty-first year, we have

achieved an increasingly mature and profound understanding of our purposes as a group dedicated to improving the well-being of those who have diabetes.

On all educational fronts we have kept up the accelerated pace of last year and in the near future there will be, I am sure, an increased tempo of our research activities.

Professionally, at this Annual Meeting five Scientific Sessions are again scheduled and an even wider variety of papers is being presented. The Ninth Postgraduate Course in New Orleans, at the beginning of the year, was well received and the Tenth Postgraduate Course to be held in Detroit next January will mark a decade of our postgraduate activity.

Our journal DIABETES next January will celebrate its tenth year of existence with as high or higher level of scientific interest and variety as ever before. In the offing are: a third edition of the *Diabetes Guide Book for the Physician*, regional Postgraduate Courses, research symposium, modification of basic diets for diabetics, a check list for teaching diabetic patients, and a new scientific exhibit.

For the patient, and those dealing directly with him, articles have been prepared and should be published in the near future, for a national teaching journal, *The American Journal of Correction*, and the somewhat monumental project of a manual for diabetics.

A brochure entitled "Diabetics Are Desirable Workers" directed primarily to management and personnel people in industry will go to press this summer.

For Diabetes Week this year, the leaflet "Diabetes-Check Facts" will be completely revised into a four-page, single fold format suitable both for general distribution and with changed text on the last page for insertion in Dreypak envelopes.

A new animated sixty- and twenty-second television spot announcement is being readied and a similar, but slightly changed version will be available for general use throughout the year thereafter.

Plans are going forward to make available at cost a Dreypak dispenser which can be placed on drug store counters and so forth.

Authorization has been given for the preparation, if satisfactory copy can be worked out, of a fifteen-minute recorded radio show for local use about the importance of recognition and understanding of insulin shock by law enforcement and correction officers as well as the public. In addition, we are cooperating with the American Correctional Association in setting up four diabetes screening programs in correctional institutions in various parts of the country.

For year-round and long-term use we are also working on a fifteen-minute public education film that will be suitable for television purposes.

Of particular interest, I know, to all members will be the fact that the long-awaited *Membership Directory* is at press.

Now, if I may, I would like to make several general observations. Although major amendments were made to our Bylaws at this Council meeting which provide for nomination by the Assembly of State Governors and Affiliate Delegates and election by the Membership at annual business meetings of five physician members of the Assembly to be placed on the Council, our organizational structure is constantly under review even after twenty-one years since we never wish to be frozen in any one pattern. We must continue to try to fulfill the basic objectives of the organization and to meet the needs and demands that are made upon us. We have grown surely—but not too slowly—as a dynamic group without resorting to undignified publicity or objectionable fund-raising technics. There is no relationship between a sound educational program and such undesirable practices. Not infrequently has our material and approach been appropriated by other organizations. We have every reason to be proud of our efforts.

Within the last year there has been considerable reference to "closing the gap" between advances in medical science and their application to the patient. We all know that this gap has always existed, and should remember with satisfaction that its presence was one of the major reasons for the organization of the American Diabetes Association in 1940.

Actually it is an ancient problem, despite the recent headlines giving the impression that this is something new. We know that it is not, and I am sure that the Association will continue to emphasize quick utilization of new knowledge in the field of the treatment of diabetes and, hopefully, its prevention.

I regret that I feel it is necessary to bring up the matter of finances. However, with an ever-increasing program and the large demands being made upon us, our costs are being pushed beyond a relatively fixed income. We must look to present sources for increased support if we are to maintain our basic agreed-upon objectives, and we must also find support through every possible new source that is acceptable to us. As in the business world, so with us, to stand still is to retreat.

And now to a more cheerful note. Once every four years it is our pleasure and honor to have the Annual Meeting in New York City, and this, of course, is one of those years. At this time I want to extend to all of you a cordial invitation to visit the office "around the corner" more or less from this hotel, and meet the members of the staff, some of whom probably have been only names or initials at the bottom of letters. I myself am going to be busy with organizational matters here at the Commodore until Tuesday noon, but most of the rest of the staff will be at the office after today. They will be delighted to see you.

Finally, if I can be of any assistance to you right now, I will be in the corridor outside this room for an hour or so after this session. I will be very glad, indeed, to try to answer any questions concerning our organizational affairs, or on any other matters on which I might possibly be helpful.

To the staff, many of whom have been serving this meeting and to the rest of the staff who have been maintaining the office, I wish to extend my great appreciation. This group of dedicated and capable people, I am sure, can not be equalled anywhere.

My report, of course, could not be concluded without my most sincere expression of gratitude to the Officers, Councilors, Committee Chairmen and members, and indeed to the entire membership for the support and great effort which has been put forth during this last year, and to Dr. Peck, as retiring President, I wish to thank him personally for his understanding and guidance as well as for his capable and inspiring administrative leadership.

J. RICHARD CONNELLY

PRESIDENT PECK: Thank you, Dick. We all appreciate your comments, and in return I think it is difficult or impossible to express the appreciation of the Officers, the Council and everyone concerned for the dedicated efforts of this entire group. As Dick says, there is nothing else like it. I don't know how he has been successful in assembling the staff that he has. They are all wonderful people and we all thank them individually and collectively.

Report of the Nominating Committee

The Nominating Committee is pleased to report the following slate:

For President, Blair Holcomb, M.D., of Portland, Oregon; for First Vice President, Jerome W. Conn, M.D., of Ann Arbor; for Second Vice President, Thomas P. Sharkey, M.D., of Dayton, Ohio; for Secretary, Laurentius O. Underdahl, M.D., of Rochester, Minnesota; for Treasurer, Edwin W. Gates, M.D., of Niagara Falls.

For Councilors, for term ending in 1964: Joseph H. Crampton, M.D., of Seattle; Leo Goodman, M.D., of Fresno, California; Robert C. Hardin, M.D., of Iowa City; Edgar A. Haunz, M.D., of Grand Forks, North Dakota; Rachmiel Levine, M.D., of New York; Christopher J. McLoughlin, M.D., of Atlanta.

The Committee also RECOMMENDS the following nominations to fill the unexpired terms ending in 1962, which will exist with the election of Laurentius O. Underdahl, M.D., and Edwin W. Gates, M.D., as Secretary and Treasurer respectively: William R. Jordan, M.D., Richmond, Virginia; Edwin L. Rippy, M.D., Dallas.

JOHN A. REED, M.D., *Chairman*
ALEXANDER MARBLE, M.D.
FRANCIS D. W. LUKENS, M.D.

(*It was moved, seconded and voted that the nominations be closed. All nominees were duly elected.*)

PRESIDENT PECK: We have now a further procedure in these nominations which has been brought about by the very recent adoption of some new Bylaws and Constitution changes. This will not occur next year but owing to the timing of the components concerned it is necessary for us to have an additional elective procedure this year.

The reason for this is that different parts of the organization met at different times and with the adoption of the new Bylaws, the way is finally open for the increased membership on the Council of representatives from the Assembly of Delegates and the Board of State Governors.

I think you have all been aware of these steps, which were initiated intensively during Dr. Lukens' administration, and this new action is the finalization of over two years of work. I now call upon the Secretary to present this part of the elective procedure.

SECRETARY SHERIDAN: Mr. President, in accordance with the new Bylaws, the Assembly of State Governors and Affiliate Delegates proposes the following names for Councilors to the membership of the Association: for one year, Maurice Protas, M.D., of Washington, D.C.; for one year, James B. Hurd, M.D., of Chicago; for one year, W. Bernard Yegge, M.D., of Denver; for two years, Elton R. Blaisdell, M.D., of Portland, Maine; for three years, Carlisle Morse, M.D., of Louisville, Kentucky.

(*It was moved, seconded and voted that the nominations be closed. All nominees were duly elected.*)

DR. LUKENS: Mr. President, before you proceed, may I bring up what must be an obvious point. A new Secretary has been elected. Our present Secretary is retiring at his request after a long and exceedingly fine term of service; and if it is not out of order I would like to propose a rising vote of thanks to Paul Sheridan.

Installation of Incoming President

FRANKLIN B. PECK, SR., M.D., *retiring President*: The past action has been a concrete example and evidence of the fact that no President ever carries out all his projects. We have now finalized a project which was started previously in Dr. Lukens' term of office. There are still other projects which are "in the mill" and of which you will become aware, I am sure, during Dr. Holcomb's 1961-1962 term.

This sensation of relinquishing responsibility is a most enjoyable one. I am very happy to have the opportunity of turning over the Banting Gavel, which is the badge of office of this organization, to you, Dr. Holcomb, as I install you as the President of the American Diabetes Association.

I can assure you from personal experience that you will find ready, willing and able hands to assist you over the coming year. I am sure that this is true of your Executive Committee, your Council, your staff and the members at large.

All those of us who have worked with you have every reason to carry in our hearts our kindest esteem and affection and our complete confidence that you will do a splendid job.

Remarks of newly installed President

Thank you very much, Dr. Peck, Mr. Past President. We have a full afternoon so all I shall say to you is that I appreciate very much the confidence you have shown in electing me to this office, even though I live 3,000 miles away from New York City, our essential office.

I assure you that this will not prevent me from giving everything I have to the continued success of this organization. I know the Council members very well, and I have great confidence in their continued dedication to the objectives of this organization.

This concludes the Business Meeting. Without further ado, the Scientific Session is now in session.

BLAIR HOLCOMB, M.D.

Tenth Postgraduate Course

Detroit and Ann Arbor, Michigan
Jan. 17-19, 1962

Entitled "Diabetes in Review: Clinical Conference, 1962," the Tenth Postgraduate Course of the American Diabetes Association will be held in Detroit and Ann Arbor, Michigan, Jan. 17, 18, and 19, 1962. Open to Doctors of Medicine, the Course was developed by the ADA Committee on Professional Education. It will be presented in cooperation with the University of Michigan Medical School, Wayne State University College of Medicine, the Wayne County Medical Society, and the Michigan Diabetes Association.

Thaddeus S. Danowski, M.D., Pittsburgh, is Chairman of the Committee and Director of the Course. Frank S. Perkin, M.D., Detroit, is Chairman of the Local Committee. The January 17 and 19 sessions will be held at The Statler Hilton hotel in Detroit, and the January 18 session at the University of Michigan in Ann Arbor.

REGISTRATION: A Preliminary Program which includes an application form is being mailed to all members of the Association and to subscribers of DIABETES. *This form should be filled out and mailed together with the fee as soon as possible to the American Diabetes Association, Inc., 1 East 45th Street, New York 17, N.Y. Applications will be accepted in the order received and registrations officially confirmed.*

FEES: The registration fee for the three-day Course is $40 for members of the American Diabetes Association and $75 for nonmembers. The full fee must be paid at the time of filing application. It will be returnable by the Association to any registrant who submits his withdrawal in writing no later than Jan. 5, 1962.

Fellows, residents, graduate students and interns in medicine and allied sciences *in schools and hospitals in the Detroit and Ann Arbor area may attend the Scientific Sessions without charge. They should register by January 5. Medical students in the Detroit and Ann Arbor area may register without charge after January 5 if space permits.*

HOTEL ACCOMMODATIONS: The Statler Hilton will serve as the headquarters hotel. Reservations cards will be sent to registrants.

POSTGRADUATE CREDIT: The American Academy of General Practice will give nineteen hours' Postgraduate Credit for the Course (Category II).

PRELIMINARY PROGRAM

WEDNESDAY MORNING, JANUARY 17
The Statler Hilton

9:00 Welcome: Blair Holcomb, President
T. S. Danowski, Chairman, Committee on Professional Education, and Director of Course
Frank S. Perkin, Chairman, Local Committee
I. FUNDAMENTAL CONSIDERATIONS
Harvey C. Knowles, Jr., Chairman

9:10 Diabetic Defects at the Cellular Level—Rachmiel Levine

9:40 Actions of Insulin and Other Hormones on Carbohydrate Metabolism of Muscle—C. R. Park

10:10 *Intermission*

10:25 Actions of Insulin in the Liver—Max Miller

10:45 Effects of Insulin and Other Hormones on Metabolism of Fat—Albert E. Renold

11:15 Factors Regulating Insulin Release—Arnold Lazarow

11:45 to 12:30 Question and Answer Period by Essayists—Rachmiel Levine, Moderator; C. R. Park, Max Miller, Albert E. Renold, Arnold Lazarow

WEDNESDAY AFTERNOON, JANUARY 17
II. PATHOGENESIS
Laurence F. Segar, Chairman

2:00 Newer Findings in Experimental Diabetes—Francis D. W. Lukens

2:30 Possible Variables in Genesis of Human Diabetes—Charles H. Best

3:00 *Intermission*

3:15 Natural History and Course of Juvenile and Adult Diabetes—Harvey C. Knowles, Jr.

3:45 The Significance of the Early Recognition of the Diabetic State—Stefan S. Fajans

4:15 to 5:15 PANEL DISCUSSION: DIABETES AND PREDIABETES PATHOGENESIS AND PREVENTION—Francis D. W. Lukens, Moderator; Charles H. Best, Harvey C. Knowles, Jr., Stefan S. Fajans and Rachmiel Levine

THURSDAY MORNING, JANUARY 18
University of Michigan, Ann Arbor

9:30 Welcome: W. N. Hubbard, Jr., Dean, University of Michigan Medical School
III. INSULIN ASSAY—ANTAGONISM AND ACIDOSIS
Jerome W. Conn, Chairman

9:40 Current Applications of Insulin Assay—Albert E. Renold

10:10 Insulin Resistance and Antagonism—George J. Hamwi

10:40 *Intermission*

10:55 Derangements in Diabetic Acidosis—T. S. Danowski

11:20 Treatment of Diabetic Acidosis—R. D. Johnson

11:45 to 12:00 Question and Answer Period by Essayists—T. S. Danowski, Moderator; Albert E. Renold, George J. Hamwi, R. D. Johnson

THURSDAY AFTERNOON, JANUARY 18
IV. RELATED METABOLIC PROBLEMS
Max Miller, Chairman

2:00 Lipoatrophic Diabetes—A Rare but Informative Disease—Jerome W. Conn
2:30 Uses of Sulfonylurea Drugs in Hypoglycemic States—Stefan S. Fajans
3:00 Glucagon—Fundamental Considerations, Diagnostic and Therapeutic Uses—Fred W. Whitehouse
3:30 to 4:30 Tour of Medical Center and Campus by bus with return to Detroit at conclusion of tour

FRIDAY MORNING, JANUARY 19

The Statler Hilton

V. TREATMENT OF DIABETES MELLITUS

Frank S. Perkin, Chairman

9:00 Low Fat and Other Diets—Laurance W. Kinsell
9:30 Insulin, Its Use and Abuse—Louis K. Alpert
10:00 *Intermission*
10:15 to 11:15 PANEL DISCUSSION: PROBLEMS IN MANAGEMENT OF BRITTLE DIABETES—Alexander Marble, Moderator; Laurance W. Kinsell, Louis K. Alpert, Kelly M. West
11:15 Oral Hypoglycemic Agents—Alexander Marble
11:45 to 12:30 PANEL DISCUSSION: PREGNANCY AND DIABETES—Blair Holcomb, Moderator; Joseph H. Crampton, Priscilla White, George C. Thosteson

LUNCHEON MEETING

(*by individual subscription*)

12:30 to 2:00 Luncheon Address: Richard J. Bing
Myocardial Metabolism in Diabetes—Dr. Bing, Chairman, Department of Medicine, Wayne State University College of Medicine

FRIDAY AFTERNOON, JANUARY 19

VI. COMPLICATIONS OF DIABETES MELLITUS

Arthur R. Colwell, Sr., Chairman

2:00 Vascular Disease in Diabetes—Philip M. LeCompte
2:30 Specificity of Vascular Lesions in Diabetes—Sidney Goldenberg
2:50 Pathology of Eye in the Diabetic—J. M. B. Bloodworth, Jr.
3:10 *Intermission*
3:25 PANEL: COMPLICATIONS OF DIABETES MELLITUS—Max Miller, Moderator; Sidney Goldenberg, J. M. B. Bloodworth, Jr.
4:15 Present and Future Research Problems in Diabetes Mellitus—Charles H. Best

FACULTY

LOUIS K. ALPERT, M.D., *Professor of Medicine, The George Washington University School of Medicine; Director, Diabetes and Endocrine Clinics, The George Washington University Hospital, Washington, D.C.*

CHARLES H. BEST, C.B.E., M.D., F.R.S., *Professor of Physiology; Director and Professor, Banting and Best Department of Medical Research, University of Toronto, Toronto, Ontario, Canada.*

RICHARD J. BING, M.D., *Chairman, Department of Medicine, Wayne State University College of Medicine, Detroit, Michigan.*

J. M. B. BLOODWORTH, JR., M.D., *Professor of Pathology; Chief, Division of Pathologic Anatomy, Ohio State University College of Medicine, Columbus, Ohio.*

JEROME W. CONN, M.D., *Professor of Medicine and Director, Metabolism and Research Unit, University of Michigan Medical School; Chief, Department of Endocrinology and Metabolism, University Hospital, Ann Arbor, Michigan.*

JOSEPH H. CRAMPTON, M.D., *Clinical Professor of Medicine, University of Washington School of Medicine; Chief, Section on Metabolism and Endocrinology, The Mason Clinic, Seattle, Washington.*

T. S. DANOWSKI, M.D., *Professor of Research Medicine, University of Pittsburgh School of Medicine, Pittsburgh, Pennsylvania.*

STEFAN S. FAJANS, M.D., *Professor of Internal Medicine, Division of Endocrinology and Metabolism, University of Michigan Medical School, Ann Arbor, Michigan.*

SIDNEY GOLDENBERG, M.D., *Senior Instructor, St. Louis University School of Medicine; Research Associate, Institute of Experimental Pathology, Jewish Hospital of St. Louis, St. Louis, Missouri.*

GEORGE J. HAMWI, M.D., *Professor of Medicine, Head, Division of Endocrinology and Metabolism, Ohio State University College of Medicine; Head, Section of Endocrinology and Metabolism, University Hospital, Columbus, Ohio.*

BLAIR HOLCOMB, M.D., *Clinical Professor of Medicine, University of Oregon Medical School; Staff Physician, Good Samaritan Hospital, Portland, Oregon.*

R. D. JOHNSON, M.D., *Associate Professor of Internal Medicine, Division of Endocrinology and Metabolism, University of Michigan Medical School, Ann Arbor, Michigan.*

LAURANCE W. KINSELL, M.D., *Director, Institute for Metabolic Research, Alameda County Medical Institutions, Oakland, California.*

HARVEY C. KNOWLES, JR., M.D., *Professor of Medicine, University of Cincinnati College of Medicine, Cincinnati, Ohio.*

ARNOLD LAZAROW, M.D., Ph.D., *Professor and Head, Department of Anatomy, University of Minnesota Medical School, Minneapolis, Minnesota.*

PHILIP M. LECOMPTE, M.D., *Associate Clinical Professor of Pathology, Harvard Medical School; Pathologist, Faulkner Hospital, Boston, Massachusetts.*

RACHMIEL LEVINE, M.D., *Chairman and Professor, Department of Medicine, New York Medical College; Director, Medical Services, Flower and Metropolitan Hospitals, New York, New York.*

FRANCIS D. W. LUKENS, M.D., *Professor of Medicine and Director, George S. Cox Medical Research Institute, University of Pennsylvania, Philadelphia, Pennsylvania.*

ALEXANDER MARBLE, M.D., *Assistant Clinical Professor of*

Medicine, Harvard Medical School; Joslin Clinic and New England Deaconess Hospital, Boston, Massachusetts.

MAX MILLER, M.D., *Associate Professor of Medicine, Western Reserve University School of Medicine, Cleveland, Ohio.*

C. R. PARK, M.D., *Professor of Physiology, Vanderbilt University School of Medicine, Nashville, Tennessee.*

ALBERT E. RENOLD, M.D., *Assistant Professor of Medicine, Harvard Medical School; Director, Baker Clinic Research Laboratory, Boston, Massachusetts.*

GEORGE C. THOSTESON, M.D., *Associate Clinical Professor of Medicine, Wayne State University College of Medicine; Physician, Harper Hospital, Detroit, Michigan.*

KELLY M. WEST, M.D., *Special Assistant Scientific Affairs, Office of International Research, National Institutes of Health, Bethesda, Maryland.*

PRISCILLA WHITE, M.D., *Assistant Professor of Pediatrics, Tufts University Medical School; Physician, Joslin Clinic and New England Deaconess Hospital, Boston, Massachusetts.*

FRED W. WHITEHOUSE, M.D., *Associate Physician, Henry Ford Hospital, Detroit, Michigan.*

WINNERS OF 1960-61 GRADUATE AND MEDICAL STUDENT-INTERN ESSAY CONTESTS

Thomas F. Necheles, M.D., Chicago, and Richard J. Mahler, M.D., New York, have been selected by the Committee on Scientific Awards of the American Diabetes Association as the winners of the 1960-61 (ninth) Graduate and Medical Student-Intern Essay Contests. Roland K. Molinet, M.D., New York, received Honorable Mention.

First prize of $250 has been presented to Dr. Necheles for the best paper submitted in the field of diabetes reporting original work, whether laboratory investigation or clinical observation. Dr. Mahler received the $100 prize for the best paper in the category of review article or case report. Dr. Molinet received Honorable Mention in the latter category. All three prize-winners were also given one-year subscriptions to DIABETES for Volume 10, 1961.

The prize-winning paper by Dr. Necheles is entitled "Some in Vitro Effects of Insulin and Thyroxine on Protein and Nucleic Acid Synthesis in Rabbit Bone Marrow." Dr. Necheles was born in 1933 in Hamburg, Germany, and is a naturalized United States citizen. He graduated from the University of Chicago in 1952, and received his medical degree in 1961. He also has a Ph.D. in physiology. From 1953 to 1956 he served in the United States Army Medical Corps. He belongs to Sigma Xi, the American Association for the Advancement of Science, American Federation for Clinical Research, Nu Sigma Nu, and Beta Theta Pi. At present he is a medical intern in the New England Medical Center, Boston. He is married and has three children.

Dr. Mahler's paper is entitled "The Relationship of Fatty Acid Metabolism to Diabetes Mellitus." He was born in 1934 in New York City and graduated from New York University in 1955. He received his medical degree from New York Medical College in 1959, and interned at New Rochelle Hospital, New Rochelle, New York. At present he is in his second year of residency in internal medicine at Flower and Fifth Avenue Hospitals, Metropolitan Medical Center, New York. He is married and resides in New York.

DR. NECHELES

DR. MAHLER

Dr. Molinet, whose paper is titled, "The Relationship of Glomerulosclerosis to Primary (Familial) and Secondary Diabetes," was born in Freeport, Long Island, and graduated from Yale University in 1955. He received his M.D. from New York Medical College in 1959. After a year of rotating internship in Good Samaritan Hospital, West Palm Beach, Florida, he returned to New York and is presently in his second year of residency in internal medicine at New York Medical College, Metropolitan Medical Center. He is married and has one child.

1961-62 GRADUATE AND MEDICAL STUDENT-INTERN ESSAY CONTESTS

Members of the American Diabetes Association and subscribers to DIABETES are asked to encourage the following to enter the tenth Graduate and Medical Student-Intern Essay Contests: medical students, interns, residents throughout the period of their residency, physicians within two years after their graduation from medical school, and graduate students in the basic sciences.

$250 Prize—for the best paper reporting original work, whether laboratory investigation or clinical observation.

$100 Prize—for the best review article or case report.

The prize-winners as well as those receiving honorable mention also are given a one-year subscription to DIABETES: *The Journal of the American Diabetes Association.*

The papers will be judged on the basis of the value of the material and method of presentation. Any subject relating to diabetes and basic metabolic problems may be selected.

Papers entered in the Contests should not have been previously published. Appropriate manuscripts will be considered for publication in DIABETES.

Contestants should submit the original and two copies of their papers. Typewritten and double-spaced, all manuscripts should be mailed with a letter of transmittal by March 15, 1962, to: Committee on Scientific Awards, American Diabetes Association, Inc., 1 East 45th Street, New York 17, N.Y.

22nd ANNUAL MEETING

As previously announced, the Twenty-second Annual Meeting of the American Diabetes Association will be held in Chicago, June 23-24, 1962, at The Conrad Hilton hotel, prior to the annual session of the American Medical Association.

Physicians and other scientists who would like to present papers at the Scientific Sessions are invited to submit abstracts to Thomas P. Sharkey, M.D., Chairman of the Committee on Scientific Programs. Seven copies of the abstracts, which are requested in order to expedite review by the Committee, should be sent as soon as possible, but not later than March 1, 1962, to Dr. Sharkey in care of the national office.

New procedures for the submission of abstracts, other than the requirements stated above, were approved by the Council at the Twenty-first Annual Meeting and will be published in the November-December issue of DIABETES. They also will be included in the communication from the Chairman of the Committee on Scientific Programs which will be sent to the membership during November.

1962 LILLY AWARD DEADLINE

Dec. 1, 1961, is the deadline for receipt of nominations for the sixth Lilly Award which will be given at the Twenty-second Annual Meeting of the American Diabetes Association, June 23-24, 1962, in Chicago. This annual award is supported by Eli Lilly and Company and consists of $1,000 and a medal. The following stipulations govern the contest:

Purpose: To recognize demonstrated research in the field of diabetes, taking into consideration independence of thought and originality.

Eligibility: Any investigator in an appropriate field of work closely related to diabetes who is less than forty years of age on January 1 of the year in which the award is made. The research will not necessarily be judged in comparison to the work of more mature and experienced workers. The candidate should be a resident of the United States or Canada.

Nominations: Nominations for the award will be solicited from the members of the American Diabetes Association. Such nominations will be requested by repeated notices to be published in DIABETES. Names of nominees will be sent to the Chairman of the Committee on Scientific Awards and must be received before December 1 of the year preceding the award. The nomination should be accompanied by full information concerning the nominee's personality, training and research work. Six copies of each item should be submitted. No member may send in more than one nomination. A list of the nominee's publications, if any, and six copies of the publication or manuscript for which the award is to be given should also accompany the nomination. At the discretion of the Committee on Scientific Awards, the award may be given for work published during the year prior to December 1 of the year preceding the award. The nominee should be actively engaged at that time in the line of research for which the award is to be made.

Announcement: The name of the winner will be announced in the program of the Annual Meeting of the Association, and the award presented at that meeting. The winner, subject to the approval of the Committee on Scientific Programs, will be invited to present a paper on his work. Papers considered for the award must be submitted with the idea that they will be published in whole or in part in DIABETES if found acceptable to the Editor and/or Editorial Board. If the Committee should decide that no outstanding work has been presented for this consideration, the award will not be made.

Award: In addition to the monetary award and medal, traveling expenses will be given to make it possible for the recipient to receive his award in person at the Annual Meeting.

NOVEMBER 15 DEADLINE FOR RESEARCH FELLOWSHIP APPLICATIONS

The Committee on Research and Fellowships of the American Diabetes Association will award at least three Research Fellowships for the academic year July 1, 1962—June 30, 1963. Nov. 15, 1961, is the deadline for receipt of applications. Requests for application forms and other inquiries should be addressed to Mr. J. Richard Connelly, ADA Executive Director, at the national office. He will forward the information to the Committee.

ADA SCIENTIFIC EXHIBIT TEXT AVAILABLE

The complete text of the latest scientific exhibit of the American Diabetes Association is available without charge. Titled "Everyday Problems in Diabetes: 201 Crystal Clear Answers," the question-and-answer exhibit was displayed in New York City at the annual meeting of the American Medical Association June 25-30, 1961. It previously had been exhibited at the AMA Annual Meeting June 13-17, 1960, in Miami Beach, Florida.

The text of the exhibit includes sections on "Steps to Routine Diagnosis," "Etiology," "Heredity," "Diagnosis," "Diet," "Insulin," "Oral Agents," "Laboratory Methods," "Management," "Complications," "Juvenile Diabetes," "Pregnancy," "Drugs," and "General Guides to Therapy."

The exhibit was developed two years ago by the ADA Committee on Scientific Exhibits: Marshall I. Hewitt, M.D., Chairman; DeWitt E. DeLawter, M.D.; Alfred H. Free, Ph.D.; Robert C. Hardin, M.D.; William R. Kirtley, M.D.; Leo P. Krall, M.D.; C. J. O'Donovan, M.D.; George F. Schmitt, M.D., in collaboration with the Steering Committee of the Committee on Professional Education as follows: T. S. Danowski, M.D., Chairman; Arthur R. Colwell, Sr., M.D., Vice Chairman; Jerome W. Conn, M.D., Vice Chairman. Dr. O'Donovan has since become Chairman of the former Committee, succeeding Dr. Hewitt who continues as Vice Chairman.

The exhibit itself is now available on a loan basis to Clinical Societies of Affiliate Associations and to other medical organizations. Text is available to members of the medical profession by writing to the American Diabetes Association, 1 East 45th Street, New York 17, N.Y.

Other exhibits which are also available on a loan basis are: "Diabetes—Today and Tomorrow: The Expanding Role of the Doctor"; "Pathology of Diabetes" and "Management of Diabetes Mellitus."

DIABETES WEEK NOVEMBER 12-18

Affiliates of the American Diabetes Association and Committees on Diabetes of County and State Medical Societies are urged to prepare final plans for their Public Education and Detection programs, which in accordance with custom will be highlighted by Diabetes Week immediately preceding Thanksgiving. New this year are a Dreypak Unit Display Dispenser (holds fifty Dreypaks), two four-page leaflets (one of them to accompany Dreypaks) and a new animated TV spot announcement.

The Organizational Kit was mailed September 1, and an announcement concerning the availability of testing materials and reagents was sent out August 22. These will be followed by a Newspaper Publicity Kit and a TV-Radio Unit. Report forms will be mailed just prior to Diabetes Week.

Joseph H. Crampton, M.D., is Chairman of the Committee on Public Education and Detection. Vice Chairmen are: Louis K. Alpert, M.D., Leo Goodman, M.D., William H. Olmsted, M.D., Frank S. Perkin, M.D., and John A. Reed, M.D.

Address your requests for information concerning available services and supplies to Mr. J. Richard Connelly, Executive Director, at the national office of the Association.

NEWS OF AFFILIATE ASSOCIATIONS

The CHICAGO DIABETES ASSOCIATION will hold its Fifth Annual Symposium on Diabetes at the Offield Auditorium, Passavant Memorial Hospital, 303 East Superior Street, Chicago, on Friday, November 10, beginning at 9:00 a.m.

Speakers will include: George F. Cahill, Jr., M.D., Peter Bent Brigham Hospital, Boston; Paul E. Lacy, M.D., Washington University School of Medicine, St. Louis; G. de Takats, M.D., Marvin Cornblath, M.D., and Theodore B. Schwartz, M.D., University of Illinois Medical School; Arthur R. Colwell, Sr., M.D., and Ralph E. Dolkart, M.D., Northwestern University Medical School; Albert Dorfman, M.D., University of Chicago Medical School; and William C. Shoemaker, M.D., and Piero P. Foa, M.D., Chicago Medical School.

A round-table discussion led by speakers on the program will be held at Abbott Hall during the luncheon recess.

Registration is free for members of the Chicago Diabetes Association or the American Diabetes Association and for medical students and resident house staff members. Fee for nonmembers is $25.00.

Members of the Academy of General Practice may claim hour for hour credit in Category II.

Inquiries concerning the symposium should be addressed to The Chicago Diabetes Association, 620 North Michigan Avenue, Chicago 11, Ill.

The NEW ENGLAND DIABETES ASSOCIATION will hold a dinner meeting and panel on "Progress and Problems in Diabetes" at the Lafayette Hotel, Portland, Maine, Friday, Oct. 13, 1961, at 7 p.m. The dinner and panel will be held preceding and in conjunction with the regional meeting of the American College of Physicians.

A panel moderated by Elton R. Blaisdell, M.D., President, New England Diabetes Association, will be as follows:

Simeon Locke, M.D., Boston, will speak on "The Neurological Concomitants of Diabetes Mellitus"; Frank C. Wheelock, M.D., Boston, on "Arterial Reconstruction in the Diabetic Patient: Indications and Results"; and Samuel B. Beaser, M.D., Boston, on "Present Status of Oral Hypoglycemic Agents."

Social hour will be held from 6 to 7 p.m., with dinner at 7. The panel meeting will start at 8.

Information about the meeting and dinner reservations may be secured from Dr. Elton R. Blaisdell, 12 Deering Street, Portland, Me.

In celebration of the 40th Anniversary of the discovery of insulin, the 30th Anniversary of the founding of the New York Diabetes Association, and the 25th Anniversary of the founding of Camp NYDA, the NEW YORK DIABETES ASSOCIATION is holding its Ninth Annual All-day Symposium entitled "Insulin and Its Metabolism," on Friday, Dec. 1, 1961, at the Hotel Pierre in New York City.

The morning session, from 9:00 a.m. to 12:00 noon, will open with an address of welcome by Irving Graef, M.D., President of the New York Diabetes Association, and will be presided over by Gerald J. Friedman, M.D., Chairman of the Clinical Society. Papers to be presented in the morning session are "Chemistry of Insulin," by Arnold Lazarow, M.D., Ph.D., Minneapolis; "Mechanism of Action of Insulin," (tentative title) by DeWitt Stetten, Jr., M.D., Ph.D., Bethesda, Maryland; "Insulin Antibodies," by Rosalyn Yalow, Ph.D., and Solomon A. Berson, M.D., New York City; and "Electronmicroscopy of the Islets of Langerhans," by Paul E. Lacy, M.D., Ph.D., St. Louis.

The afternoon session, with Max Ellenberg, M.D., Chairman-Elect of the Clinical Society, presiding, will begin at 2:00 p.m. The opening paper will be "History of Insulin," by Charles H. Best, M.D., D.Sc., F.R.S., Toronto, Canada, followed by "Clinical Impact of Insulin," by Harold Rifkin, M.D., New York. A panel discussion on "The Effect of Insulin on the Liver," including Rachmiel Levine, M.D., Moderator, and Richard Charles de Bodo, M.D., New York, Leonard L. Madison, M.D., Dallas, and Sidney Weinhouse, Ph.D., Philadelphia, Panel Members, will conclude the afternoon program.

Physicians, medical students and other professional personnel are cordially invited to attend the Symposium. There is no registration fee for medical students, interns, residents, and members of the Clinical Society. Fee for all others is $5.00. Advance registration is required and may be made by calling or writing the New York Diabetes Association, 104 East 40th Street, New York 16, N.Y., OXford 7-7760. A subscription luncheon will be held at the Hotel Pierre. Admission to the meetings and luncheon will be by ticket only.

A Ball honoring Dr. Charles H. Best will be held at the Hotel Pierre on the night of December 1, at 7:30 p.m.

NEWS NOTES

ASSEMBLY IN ENDOCRINOLOGY

The Thirteenth Postgraduate Assembly in Endocrinology and Metabolism will be held under the co-sponsorship of The Endocrine Society and the National Institutes of Health, Bethesda, Maryland, Oct. 2-6, 1961. A comprehensive review of clinical endocrine problems and current research activity

in these areas will be presented. Fees are $100 for physicians, $30 for residents and fellows. For information write: Dr. Roy Hertz, National Institutes of Health, Building 10, Bethesda 14, Maryland.

ADA MEMBERS PARTICIPATE IN ACP SESSIONS

The following members of the American Diabetes Association will take part in Postgraduate Course No. 2, "The Physiologic Basis of Internal Medicine," Oct. 9-13, 1961, at Duke University Medical Center, Durham, North Carolina: MORTON D. BOGDONOFF, M.D. ("Physiology of Hyperlipemia and Hypercholesteremia," and "Physiologic Basis of Reactions of Chronic Illness"); FRANK L. ENGEL, M.D. ("Interrelations between Fat and Carbohydrate Metabolism"); and JOHN V. VERNER, M.D. ("Calcium and Phosphorus Metabolism").

At the Forty-second Annual Session of The American College of Physicians held at Bal Harbour, Miami Beach, Florida, May 8-12, 1961, these members of the ADA participated:

Clinical Session, Monday afternoon, May 8: JOHN A. COLWELL, M.D., Chicago, "Disseminated Coccidioidomycosis: Its Natural Course and Treatment."

Clinical Investigation Session, Monday, May 8: ELLSWORTH L. AMIDON, M.D., Burlington, Vermont, Presiding Officer.

Basic Medical Science Session, Monday, May 8: ROBERT H. WILLIAMS, M.D., Seattle, Presiding Officer.

Basic Medical Science Session, Tuesday, May 9: GEORGE W. THORN, M.D., Boston, Presiding Officer.

Clinical Session, Tuesday, May 9: DWIGHT L. WILBUR, M.D., San Francisco, Presiding Officer.

Clinical Investigation Session, Tuesday, May 9: ALLEN R. HENNES, M.D., Oklahoma City, "Defective Synthesis of Triglycerides from C-14 Acetate in the Well-controlled Stable Diabetic"; THOMAS G. SKILLMAN, M.D., and GEORGE J. HAMWI, M.D., both of Columbus, Ohio, "Motor Nerve Conduction Velocity in Diabetes Mellitus."

Basic Medical Science Session, Wednesday, May 10: ALBERT I. WINEGRAD, M.D., Philadelphia, "Some Important Aspects of Carbohydrate Metabolism in Fat and Protein Metabolism."

Panel Discussion 1, Tuesday, May 9, "Hypoglycemia: A Clinical Dilemma": GEORGE W. THORN, M.D., Boston, Moderator; STEFAN S. FAJANS, M.D., Ann Arbor; and ALBERT E. RENOLD, M.D., Boston, panel members.

Panel Discussion 8, Wednesday, May 10, "Growth Hormone": JOHN C. BECK, M.D., Montreal, Moderator; PHILIP H. HENNEMAN, M.D., Jersey City, panel member.

Panel Discussion 16, Friday, May 12: "Clinical Approach to the Patient with Hypercalcemia": WILLIAM G. THOMAS, JR., M.D., Gainesville, Florida, Moderator; THOMAS B. CONNOR, M.D., Baltimore, panel member.

Panel Discussion 18, Friday, May 12, "Chemotherapy of Neoplastic Diseases": SAMUEL G. TAYLOR, III, M.D., Chicago, panel member.

OMAHA MID-WEST CLINICAL SOCIETY WILL HOLD POSTGRADUATE ASSEMBLY

Oct. 30 through Nov. 2, 1961, are the dates for the Twenty-ninth Annual Postgraduate Assembly of the Omaha Mid-West Clinical Society, which will be held in Omaha's Civic Auditorium. The subject "Prediabetes in Pregnancy" will be presented October 30, and "Current Concepts in Surgery of the Pancreas" October 31. Members of the American Diabetes Association, who will participate in a panel discussion entitled "The Management of Diabetic Complications" on October 30 are: CHARLES R. HANKINS, M.D., Moderator; ROBERT S. LONG, M.D., and HENRY J. LEHNHOFF, JR., M.D.

PERSONALS

MARTIN G. GOLDNER, M.D., New York, has been appointed Director of Medicine of The Jewish Hospital of Brooklyn and Professor of Medicine of the State University of New York, Downstate Medical Center.

S. J. LONDON, M.D., New York, has been elected Vice President and Medical Director of M. R. Thompson, Inc., a subsidiary of The J. B. Williams Company. Dr. London was formerly Vice President and Medical Director of The Purdue Frederick Company.

G. E. MAHA, M.D., formerly of Indianapolis, has accepted an appointment as staff physician at the University of Illinois in Champaign.

MAXWELL SPRING, M.D., New York, will chair the program of the second annual Internist's Day sponsored by the metropolitan chapter of the New York State Society of Internal Medicine. The program will consist of a symposium on hypertension and will be held Sept. 27, 1961, at the Waldorf-Astoria Hotel in New York. Dr. Spring also presented a paper on "Automatic Electronic Blood Pressure Apparatus" at the 4th International Conference on Medical Electronics on July 20, 1961, in New York.

KELLY M. WEST, M.D., formerly of Oklahoma City, has been named Special Assistant Scientific Affairs, Office of International Research, National Institutes of Health, Bethesda, Maryland.

MICHAEL G. WOHL, M.D., Philadelphia, will be co-chairman of the afternoon session of a "Symposium on the Problem of Alcoholism in the Practice of Medicine" which will be held Oct. 30, 1961, at the College of Physicians of Philadelphia. CARROLL M. LEEVY, M.D., Jersey City, will present "Nutritional and Metabolic Aspects of Alcoholism." Dr. Wohl is Chairman of the Committee on Nutrition and Metabolism of The Philadelphia County Medical Society which is sponsoring the symposium jointly with the Division of Behavioral Problems, Department of Health, Commonwealth of Pennsylvania, and the National Vitamin Foundation.

NECROLOGY

DAVID S. GLUSKER, Whitby, Ontario, Canada, born May 8, 1904.

GERHARDT KATSCH, Greifswald, East Germany, born May 14, 1887.

KENNETH E. THORNBURG, Indianapolis, born July 23, 1905.

GEORGE A. WESTFALL, JR., Halstead, Kansas, born February 8, 1919.